

Toxicity and Bioconcentration Potential of the Agricultural Pesticide Endosulfan in Phytoplankton and Zooplankton

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Abstract. Agricultural pesticide runoff in southeastern coastal regions of the United States is a critical issue. Bioconcentration of pesticides by phytoplankton and zooplankton at the base of the aquatic food web may increase the persistence of pesticides in aquatic ecosystems and cause effects at higher trophic levels. This study examined the toxicity of a widely used agricultural pesticide, endosulfan, to *Pseudokirchneriella subcapitata* (freshwater green alga) and *Daphnia magna* (freshwater cladoceran). We then investigated the potential of both plankton species to sequester endosulfan from their surrounding media. We also assessed the degree to which endosulfan is accumulated by *D. magna* via food (endosulfan-contaminated *P. subcapitata*). A 96-h growth rate EC₅₀ of 427.80 µg/L endosulfan was determined for *P. subcapitata*, whereas a 24-h immobilization EC₅₀ of 366.33 µg/L endosulfan was determined for *D. magna*. The 5-h EC₅₀s for filtration and ingestion in *D. magna* were 165.57 µg/L and 166.44 µg/L, respectively. An average bioconcentration factor (BCF) of 2,682 was determined for *P. subcapitata* exposed to 100 µg/L endosulfan for 16 h. An average BCF of 3,278 was determined for *D. magna* in a 100 µg/L endosulfan water-only exposure. There was negligible uptake of endosulfan by *D. magna* feeding on contaminated algae in clean water (BCF ~ 0). Different proportions of parent isomers (endosulfan I and II) and the primary degradation product (endosulfan sulfate) were detected among treatments. Endosulfan was rapidly accumulated and concentrated from water by *P. subcapitata* and *D. magna* neonates. Endosulfan contained in phytoplankton, however, was not bioaccumulated by zooplankton. These findings may prove useful

in assessing ecosystem risk, because uptake from the water column appears to be the dominant route for bioconcentration of endosulfan by zooplankton.

Studies of pesticide residues in plankton have primarily addressed the persistent organochlorine insecticides (*e.g.*, DDT, dieldrin, and chlordane), most of which have been banned from use in North America. Limited information is available on residues of newer, less persistent but heavily used pesticides, such as endosulfan (Day 1990). Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide) is a commonly used cyclodiene insecticide applied to protect cotton, tobacco, coffee, cereal, fruit, and vegetable crops against a wide array of insect pests. The U.S. Geological Survey estimated that a total of 812,787 kg of endosulfan was applied in the United States during 1992 (Thelin and Gianessi 2000). Ontario, Canada, used 25,000 kg of endosulfan in 1992 on fruit and vegetable crops alone (Harris *et al.* 2000). In the Mexican coastal zone of the Gulf of Mexico, 41,755 kg of endosulfan active ingredient was applied during 1989–1990 (Benitez and Barcenas 1996). Agricultural runoff of rain and irrigation water introduces endosulfan into the aquatic environment, where it poses significant toxicological risks to resident organisms (Scott *et al.* 1990).

This chlorinated compound exists in two stereoisomeric forms, endosulfan I and endosulfan II. The molecular weight of endosulfan is 406.9 and its solubility in water is approximately 0.32 mg/L (Kidd and James 1991). The hydrolysis half-life of endosulfan is approximately 1 month (20°C, pH 7) and the oxidation half-life is about 70 days (20°C) (ATSDR 1993). Biotransformation of endosulfan has been shown to produce endosulfan sulfate, endosulfan diol, endosulfan ether, and endosulfan lactone (Callahan *et al.* 1979). Endosulfan sulfate can persist in natural water for months and can be as toxic to aquatic organisms as the parent compound (Callahan *et al.* 1979; Wan *et al.* 1995).

Compared to other groups, data regarding the toxic effects of endosulfan on plankton are limited. In addition, information is lacking regarding the potential for these organisms to bioaccu-

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mulate endosulfan. Phytoplankton and zooplankton are particularly likely to take up pesticides from water because they generally have a high surface area to volume ratio. In addition, endosulfan's lipophilic nature may allow it to partition easily from aqueous media into the lipid components of living cells (Rao and Lal 1987; Nagel and Loskill 1991). Bioconcentration by plankton could expose fish and other organisms to toxic levels of endosulfan via ingestion, even while endosulfan levels in the water column are below the toxic threshold (Schimmel *et al.* 1977).

The objectives of this study were (1) to determine the toxicity of endosulfan to the green alga *Pseudokirchneriella subcapitata* Hindak (formerly *Selenastrum capricornutum*), and the freshwater zooplankter *Daphnia magna*; and (2) to test the potential for phytoplankton to bioconcentrate endosulfan and trophically transfer the pesticide to invertebrate grazers.

Materials and Methods

Test Organisms and Culture Conditions

Culture flasks and tubes containing sterile freshwater media (ASTM 1996) were inoculated from a parent culture of *P. subcapitata* (Carolina Biological Supply, Burlington, NC). Stock cultures were kept in a Revco® Environmental Chamber at 25°C under cool-white fluorescent lighting ($86 \pm 8.6 \mu\text{E}/\text{m}^2/\text{s}$), with a 16:8 h light:dark photoperiod and continual mixing using an orbital shaker set at 1,400 rpm. Cultures were sterile-transferred as needed to maintain log phase growth.

D. magna were cultured in large glass petri dishes containing 1 L of well-aerated, reconstituted dilution water (pH 7.8–8.0, hardness 160–180 mg CaCO_3/L , alkalinity 110–120 mg CaCO_3/L) (ASTM 1996) and maintained in a Revco® Environmental Chamber at 25°C under cool-white fluorescent lighting ($86 \pm 8.6 \mu\text{E}/\text{m}^2/\text{s}$), with a photoperiod of 16 h light, 8 h dark. They were fed contaminant-free *P. subcapitata ad libitum*, and culture water was renewed weekly. Neonates less than 24 h old were used in the experiments.

Toxicity of Endosulfan to *P. subcapitata*

A standard 96-h static algal bioassay protocol (ASTM 1996) was used to determine the toxicity of endosulfan to *P. subcapitata*. Technical grade endosulfan (98.6% pure) was obtained from AgrEvo USA (Pikeville, NC). An endosulfan stock solution was prepared in 100% acetone, and the doses were administered to obtain a final acetone concentration of 0.1% in each treatment. The nominal concentrations of endosulfan tested were 0, 130, 216, 360, 600, and 1,000 $\mu\text{g}/\text{L}$. Treatments without pesticide contained an equivalent amount of acetone carrier (0.1%). Three replicate test tubes were used for each treatment, each containing 25 ml of media. Each tube was inoculated from a sterile culture flask to provide an initial cell density of approximately 20,000 cells/ml. The test was conducted under culture conditions described above, and tubes were repositioned within the environmental chamber each day to minimize possible spatial differences in illumination and temperature on growth rate.

Cell density was assessed at approximately the same time each day (0, 24, 48, 72, and 96 h) via direct counts. A minimum of 18 grids or 400 cells were counted from 100- μl aliquots on a hemacytometer. Two homogenized samples were counted per tube per time period. *P. subcapitata* growth rates were calculated based on the cell counts. The cell count data were converted by taking the log of cell counts and

then determining the slope of the log values over time. Growth rate (divisions/day) for each replicate was the product of the slope and 3.322 (Sorokin 1973). An average growth rate for each treatment was then determined. The data were analyzed using the linear interpolation method for sublethal toxicity (Norberg-King 1993), whereby a 96-h EC_{50} ($\mu\text{g}/\text{L}$) was generated.

Acute Toxicity of Endosulfan to *D. magna*

A standard 24-h static bioassay protocol (ASTM 1996) was used to determine the toxicity of endosulfan to *D. magna*. The nominal concentrations of endosulfan tested were 0, 130, 216, 360, 600, and 1,000 $\mu\text{g}/\text{L}$. Controls were exposed to an equivalent amount of acetone carrier (0.1%). Three replicate beakers were used for each treatment, each containing 10 *D. magna* neonates in 25 ml of media. The test was conducted under culture conditions described above, but *D. magna* were not fed during the exposure. A 24-h immobilization EC_{50} ($\mu\text{g}/\text{L}$) was then calculated using probit analysis software (US EPA 1992).

Sublethal Toxicity of Endosulfan to *D. magna* (Effects on Feeding Efficiency)

The effect of endosulfan on *D. magna* feeding efficiency was examined following the methods of Fernandez-Casalderrey *et al.* (1994). Neonates were placed in clean dilution water for 5 h before the test began to allow clearance of gut contents. The nominal concentrations of endosulfan tested were 0, 100, 200, 300, and 400 $\mu\text{g}/\text{L}$. Controls contained an equivalent amount of acetone carrier (0.1%). Three replicate beakers were used for each treatment, each containing 10 *D. magna* neonates in 25 ml of media. An appropriate volume of *P. subcapitata* culture was added to yield 100,000 cells/ml in each beaker. The initial *P. subcapitata* cell density was confirmed via direct cell counts from an extra control beaker. The test beakers were placed in the dark for 5 h. After 5 h the number of immobile *D. magna* and the density of *P. subcapitata* in each beaker were assessed. Average filtration (F ; ml/ind/h) and ingestion (I ; cell/ind/h) rates were calculated as follows (Gauld 1951):

$$\text{Filtration rate} = V/n ((\ln C_o - \ln C_t)/t) - A$$

$$A = (\ln C_o - \ln C_t)/t$$

$$\text{Ingestion rate} = F \sqrt{(C_o \cdot C_t)}$$

where V = volume (ml), C_o = initial food concentration (cells/ml), C_t = final food concentration (cells/ml), t = duration of the experiment in h, n = number of organisms in volume V (ml), A = correction factor for changes in the control with final concentration C_t after time t , and F = filtration rate.

Five-hour EC_{50} s for filtration and ingestion were generated using the linear interpolation method for sublethal toxicity (Norberg-King 1993).

Uptake of Endosulfan by *P. subcapitata*

Aliquots (40 ml each) of the *P. subcapitata* stock culture (3,660,000 cells/ml) were transferred into four preweighed glass centrifuge tubes. Three replicates were dosed to yield a final concentration of 100 $\mu\text{g}/\text{L}$ endosulfan. The control tube received an equivalent amount of acetone (0.1%). The test was performed under culture conditions described

above. Cell density was determined before dosing and 16 h after dosing.

After 16 h, an exposure time similar to that of Rao and Lal (1987), the algal mass was separated by centrifugation (approximately 700 g for 30 min) and the supernatant was decanted and saved for gas chromatography (GC) analysis. Approximately 40 ml of supernatant was extracted onto a C18 cartridge, eluted with ethyl acetate, and concentrated into iso-octane for later GC analysis.

The algal pellet was washed with deionized water and recentrifuged twice as before. Each algal pellet was then dissolved in 2 ml methanol and vortexed. An equal amount of hexane was then added and the contents mixed. After phase separation, a 1-ml aliquot of the hexane layer was transferred to a GC vial for analysis. An internal standard (48 mg of δ -hexachlorohexane) was added to all samples before extraction.

To estimate the mass of the algal pellet, 40-ml aliquots of *P. subcapitatum* stock culture were transferred into three preweighed glass centrifuge tubes and cells were counted. The tubes were centrifuged at 2,500 rpm for 30 min, and supernatant was then poured off and discarded. A wet weight was then determined. The tubes were placed in an oven at 80°C overnight and reweighed to determine dry weight.

Uptake of Endosulfan by D. magna

Neonates were exposed to four different treatments: (1) endosulfan-contaminated water with clean food (three replicates); (2) endosulfan-contaminated food in clean water (three replicates); (3) endosulfan-contaminated food and endosulfan-contaminated water (two replicates); and (4) a control treatment of clean water and clean food (two replicates).

Feeding cultures were prepared to provide *D. magna* neonates with either a clean or an endosulfan-contaminated food source. Two 80-ml aliquots of *P. subcapitatum* stock culture were transferred into clean flasks. One flask was dosed to yield a final nominal concentration of 100 $\mu\text{g/L}$ endosulfan. The second flask was dosed with an equivalent amount of acetone carrier (0.1%). Both were incubated under algal culture conditions for 24 h.

A nominal water concentration of 100 $\mu\text{g/L}$ endosulfan or equivalent acetone carrier and a feeding density of 206,000 algal cells/ml were used for all treatments. The density of algal cells was similar to the number of cells/*Daphnia* used in the feeding efficiency experiment. The endosulfan concentration of 100 $\mu\text{g/L}$ was selected to be \leq the no observable effects concentration (NOEC) for *D. magna* neonates, as determined in the feeding efficiency experiment.

Treatment solutions were prepared in 50-ml volumetric flasks. Approximately 20 ml dilution water was added to each flask followed by a volume of dosed or nondosed feeding culture adequate to attain a final *P. subcapitatum* cell density of 206,000 cells/ml. Then 150 μl of 100 mg/L endosulfan in acetone solution or an equal volume of acetone carrier was added, and the volume was brought to 50 ml with dilution water. Following a 5-h period in clean dilution water to allow clearance of gut contents, *D. magna* neonates were placed into beakers containing 100 ml of dilution water. A total of 62 neonates was added to each beaker. At 0 h the treatment solutions were added to each beaker to bring the total volume to 150 ml. Treatments were then incubated under *D. magna* culture conditions described above for 24 h. *D. magna* dry weight was determined using 3 replicates of 10 neonates taken from the culture. An average neonate dry weight was then calculated.

Cell counts of *P. subcapitatum* were taken at 0 h, 5 h, and 24 h to determine *D. magna* feeding rates using the methods described previously. After 24 h mobility was assessed. The contents of each beaker were poured through a microsieve to collect neonates, and the liquid was saved for extraction and GC analysis using the methods described

earlier. *D. magna* neonates were rinsed with deionized water, transferred into round-bottomed tubes and frozen at -80°C until analysis (see following section).

Endosulfan Extraction from D. magna Neonates

Tubes containing frozen neonates were allowed to reach room temperature and dried with 6 g Na_2SO_4 . The neonate tissue was homogenized in the tubes using a Teflon-tipped pestle. Dichloromethane (11 ml) and 48 mg of the volume correction standard, δ -hexachlorohexane, were then added to each tube. Each tube was vortexed and then sonicated for 30 min. The solvent was then transferred to a separate Turbopap evaporation tube and reduced to 0.5 ml under nitrogen gas using a Turbopap concentrator. Hexane (5 ml) was then added and the volume was reduced again to 0.5 ml.

The concentrated extracts were then cleaned by elution through conditioned florisil columns (Fluka Chemical, Milwaukee, WI). Each column consisted of a 10-inch Pasteur pipette containing 5 cm florisil topped with 0.5 cm Na_2SO_4 . Each column was conditioned by elution of 5 ml ethyl acetate and 10 ml hexane through the column. This was followed immediately by sample elution through the column into a clean Turbopap tube by 10 ml of 20% ethyl acetate/80% hexane. Internal standard (deuterated endosulfan I [44 mg]) was added to each tube, followed by solvent exchange to iso-octane and concentration to 1 ml. The concentrated extract was then transferred to a GC vial for analysis.

GC Quantification of Endosulfan Concentrations from Plankton Extracts

All extracts and stocks were analyzed using capillary GC-Electron Capture Detection (ECD) (Hewlett Packard 5890 series II, Avondale, PA) and quantified using an internal standard method. The extraction efficiency estimated using the internal standard was 72% (\pm 5%) for *P. subcapitatum* and 75% (\pm 7%) for *D. magna* neonates. Data were digitally collected and processed using PC-based EZChrom Elite software (Scientific Software).

A dual-column method was used for analyte separation and confirmation. The analytical columns were a DB-5 (J&W Scientific, 5% phenyl, 95% methyl polysiloxane, 30 m long; 0.25 mm internal diameter; 0.25 μm film) and an Rtx-50 (Restek Corporation Rtx-50, 50% phenyl, 50% methyl polysiloxane, 30 m; 0.25 mm internal diameter; 0.25 μm film). A 5-m deactivated guard column was connected to the capillary injection port. The guard column effluent end was connected to a Y connector, which splits the carrier gas stream. The heads of the two analytical columns were then connected to the Y connector effluent. The analytical columns each terminated with a separate ECD.

Sample injections (2 μl) were performed in splitless mode using an HP7673 autosampler. The gas chromatograph was configured as follows: column gas—helium; detector gas—argon/methane (95%/5%); inlet temperature— 220°C ; detector temperature— 350°C .

Determination of Bioconcentration Factors

Endosulfan uptake results were described in terms of bioconcentration factors (BCFs). A $\text{BCF}_{(\text{water})}$, the ratio of chemical concentration in the organism to that of the water, was calculated for *P. subcapitatum* and *D. magna*. A $\text{BCF}_{(\text{food})}$, the amount of chemical assimilated into tissue via food, was determined for *D. magna* treated with contaminated algae in clean water. For *D. magna* treated with contaminated algae in contaminated water, a $\text{BCF}_{(\text{water} + \text{food})}$ was determined. The following

equations describe how endosulfan uptake was determined, and assume steady state equilibrium.

P. subcapitatum: Endosulfan bioconcentrated from water:

$$\text{BCF}_{(\text{water})} = (P/C)/W$$

where P = total endosulfan detected in algal pellet (ng), C = algal pellet dry weight (mg), and W = nominal endosulfan concentration in water (ng/mg).

D. magna: Endosulfan bioconcentrated from water:

$$\text{BCF}_{(\text{water})} = (T/D)/W$$

Endosulfan bioconcentrated from food:

$$\text{BCF}_{(\text{food})} = (T/D)/M$$

Endosulfan bioconcentrated from water and food:

$$\text{BCF}_{(\text{water} + \text{food})} = (T/D)/W + (T/D)/M$$

where T = total endosulfan detected in neonate tissue (ng), D = neonate dry weight (mg) = (number of live neonates multiplied by average dry weight [mg] of 1 neonate), W = nominal endosulfan concentration in water (ng/mg), and M = concentration of endosulfan (ng/mg) in algae consumed = ([ng endosulfan/algal cell multiplied by total number algal cells ingested]/average dry weight [mg] of algal cell).

Results

Toxicity Experiments

A 96-h growth rate EC_{50} of 427.80 $\mu\text{g/L}$ (95% confidence limits: 283.32–510.43 $\mu\text{g/L}$) was determined for *P. subcapitatum*. The NOEC for *P. subcapitatum* growth was 130 $\mu\text{g/L}$ endosulfan. A 24-h immobilization EC_{50} of 366.33 $\mu\text{g/L}$ (95% confidence limits: 228.67–514.67 $\mu\text{g/L}$) was determined for *D. magna*. The 5-h EC_{50} s for filtration and ingestion in *D. magna* were 165.57 $\mu\text{g/L}$ (95% confidence limits: 139.12–233.79) and 166.44 $\mu\text{g/L}$ (95% confidence limits: 138.25–218.66), respectively. The NOEC determined for *D. magna* feeding efficiency was 100 $\mu\text{g/L}$ endosulfan.

Uptake of endosulfan by *P. subcapitatum*

P. subcapitatum readily accumulated and concentrated endosulfan from the surrounding water. An average total mass of 1,029.7 ng endosulfan was measured in the algal pellets, whereas an average total mass of 92.5 ng endosulfan was detected in the water after 16 h (Table 1). Endosulfan was not detected in the control algae or control water (Table 1). The average pellet dry weight was 3.84 mg, and the average number of cells in each sample after 16 hours was 1.95×10^8 . We estimated an average concentration of 5.28×10^{-6} ng endosulfan/cell, or 268.2 ng endosulfan/mg algae. The average BCF calculated for *P. subcapitatum* was 2682.

In algal tissue, endosulfan I comprised 76.7% of the total endosulfan recovered, and endosulfan II and endosulfan sulfate

accounted for 13.7 and 9.6%, respectively (Table 1). The water contained a smaller proportion of endosulfan I (52.9% of the total), and approximately twice as much endosulfan II (28.5% of the total) and endosulfan sulfate (18.8% of total) as the algal tissue (Table 1). The technical-grade endosulfan stock solution contained 67% endosulfan I and 33% endosulfan II.

Uptake of Endosulfan by *D. magna*

D. magna also readily accumulated endosulfan from the surrounding water, but not from the contaminated algae. Amounts of endosulfan detected in *D. magna* neonate tissue were highest in treatments that included endosulfan-contaminated water (Table 2). Neonates in the dosed water–clean algae treatment contained an average of 327.8 ng/mg endosulfan, and neonates in the dosed water–dosed algae treatment contained a similar concentration of 312.7 ng/mg. The dosed algae–clean water treatment contained only 8.4 ng/mg endosulfan (Table 2). Based on the average number of algal cells ingested in the dosed algae–clean water treatment during the 24-h exposure (18×10^6 cells), and the average concentration of endosulfan in the algal cells at the start of the exposure (5.28×10^{-6} ng endosulfan/cell), the *Daphnia* in this treatment should have ingested an average of 95.9 ng endosulfan (274.4 ng/mg *Daphnia* tissue). Instead, only an average of 8.4 ng/mg endosulfan was detected in that treatment (Table 2).

In both the neonates (Table 2) and in the water (Table 3), levels of the parent isomers were generally much higher than levels of endosulfan sulfate. The exception was the clean water–dosed algae treatment, where endosulfan sulfate in the water comprised approximately 71% of the total, compared to 3.3% and 5.0% in the other treatments (Table 3).

D. magna neonates accumulated a greater percentage of endosulfan II in the dosed water–dosed algae treatment compared to the dosed water–clean algae treatment (Table 2). Water from the dosed water–dosed algae treatments also contained greater proportions of isomer II than I (Table 3). Endosulfan II made up 59% of the total endosulfan recovered from the tissue (Table 2) and 78% of the total recovered from the water in the dosed water–dosed algae treatment (Table 3), compared to 39% (Table 2) and 50% (Table 3), respectively, in the dosed water–clean algae treatment.

Using neonate dry weight, an average $\text{BCF}_{(\text{water})}$ of 3,278 was calculated from the dosed water–clean algae treatment. The endosulfan contribution from food (clean water–dosed algae treatment) was negligible ($\text{BCF}_{(\text{food})} = 2.13 \times 10^{-9}$). The $\text{BCF}_{(\text{water} + \text{food})}$ calculated for the dosed water–dosed algae treatment was 3,127, very similar to the $\text{BCF}_{(\text{water})}$.

Discussion

The 96-h growth rate EC_{50} determined for endosulfan using *P. subcapitatum* (427.80 $\mu\text{g/L}$) is within the range of endosulfan concentrations reported to inhibit growth in other algal species. *Anabaena* sp. and *A. fertilissima* growth rates were reduced by 51.7% and 37.7%, respectively, at 1 mg/L endosulfan (Lal and Lal 1988). In the marine red alga *Champia parvula*, endosulfan concentrations of 47 and 130 $\mu\text{g/L}$ chronically impaired female

Table 1. Amount of endosulfan measured from *P. subcapitatum* algal pellet and treatment water after 16 h (water dosed at 100 µg/L endosulfan)

| | | Control Algal Pellet | Control Water | Dosed Algal Pellet | Dosed Water |
|-------------------------------|--------------|-------------------------|------------------|-----------------------|----------------|
| Endosulfan I (total ng) | Average (SE) | 0.0 (0.0) | 0.0 (0.0) | 789.7 (111.2) | 48.8 (3.4) |
| | % of total | 0.0 | 0.0 | 76.7 | 52.9 |
| Endosulfan II (total ng) | Average (SE) | 0.0 (0.0) | 0.0 (0.0) | 141.3 (20.0) | 26.3 (3.5) |
| | % of total | 0.0 | 0.0 | 13.7 | 28.5 |
| Endosulfan sulfate (total ng) | Average (SE) | 0.0 (0.0) | 0.0 (0.0) | 98.7 (6.7) | 17.3 (1.5) |
| | % of total | 0.0 | 0.0 | 9.6 | 18.8 |
| Total endosulfan (total ng) | Average (SE) | 0.0 (0.0) | 0.0 (0.0) | 1,029.7 (131.2) | 92.5 (7.1) |
| Total endosulfan (ng/mg) | Average (SE) | 0.0 (0.0) | 0.0 (0.0) | 268.2 (63.8) | 2.3 (0.2) |

Table 2. Amount of endosulfan measured in *D. magna* neonate tissue after 24 h (water and algae dosed at 100 µg/L endosulfan)

| Neonate Tissue | | Clean Water– Clean Algae | Dosed Water– Clean Algae | Clean Water– Dosed Algae | Dosed Water– Dosed Algae |
|-------------------------------|--------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Endosulfan I (total ng) | Average (SE) | 0.0 (0.0) | 66.1 (29.8) | 0.8 (0.4) | 29.1 (7.8) |
| | % of total | 0.0 | 56.7 | 31.0 | 34.2 |
| Endosulfan II (total ng) | Average (SE) | 0.0 (0.0) | 45.0 (10.5) | 1.4 (0.7) | 50.3 (0.0) |
| | % of total | 0.0 | 38.6 | 50.6 | 59.1 |
| Endosulfan sulfate (total ng) | Average (SE) | 0.0 (0.0) | 5.5 (2.8) | 0.5 (0.3) | 5.7 (1.1) |
| | % of total | 0.0 | 4.7 | 18.4 | 1.1 |
| Total endosulfan (total ng) | Average (SE) | 0.0 (0.0) | 116.6 (37.8) | 2.8 (0.9) | 85.1 (8.9) |
| Total endosulfan (ng/mg) | Average (SE) | 0.0 (0.0) | 327.8 (115.7) | 8.4 (2.9) | 312.7 (18.6) |

Table 3. Amount of endosulfan measured in *D. magna* treatment water after 24 h (water and algae dosed at 100 µg/L endosulfan)

| Treatment Water | | Clean Water– Clean Algae | Dosed Water– Clean Algae | Clean Water– Dosed Algae | Dosed Water– Dosed Algae |
|-------------------------------|--------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Endosulfan I (total ng) | Average (SE) | 0.4 (0.4) | 464.3 (136.1) | 2.6 (0.7) | 176.0 (74.0) |
| | % of total | 100 | 49.6 | 15.5 | 17.4 |
| Endosulfan II (total ng) | Average (SE) | 0.0 (0.0) | 441.0 (141.3) | 2.3 (1.1) | 786.5 (90.5) |
| | % of total | 0.0 | 47.1 | 13.8 | 77.6 |
| Endosulfan sulfate (total ng) | Average (SE) | 0.0 (0.0) | 31.2 (5.5) | 11.8 (0.4) | 50.8 (3.3) |
| | % of total | 0.0 | 3.3 | 71.0 | 5.0 |
| Total endosulfan (total ng) | Average (SE) | 0.4 (0.3) | 936.5 (54.6) | 16.7 (0.6) | 1,013.3 (167.8) |
| Total endosulfan (ug/L) | Average (SE) | 0.0 (0.0) | 18.7 (1.1) | 0.3 (0.0) | 20.3 (3.4) |

growth and tetrasporophytes, respectively. Higher levels, 360–600 µg/L, produced chronic reproductive effects in the alga. The maximum allowable toxicant concentration determined for endosulfan in *C. parvula* was < 47 µg/L (Thursby *et al.* 1985). Mohapatra and Mohanty (1992) found endosulfan to be more toxic to the cyanobacterium *Anabaena doliolum* (10-day LC₅₀ of 2.15 mg/L) than to the green alga *Chlorella vulgaris* (10-day LC₅₀ of 41.5 mg/L). A delayed inhibition of growth exhibited by *Chlorella* sp. was thought to be due to efficient accumulation and concentration of endosulfan inside the cell (Mohapatra and Mohanty 1992).

In this study, endosulfan was found to affect feeding behavior of *D. magna* at concentrations half as high (166 µg/L) as the 24-h immobilization EC₅₀ of 366.33 µg/L. Fernandez-Casalderrey *et al.* (1994) also found *D. magna* feeding efficiency to be reduced at endosulfan concentrations below their 24-h LC₅₀, using the unicellular algae *Nannochloris oculata* (24-h LC₅₀ of 620 µg/L, 5-h EC₅₀s for filtration and ingestion of 440 µg/L and 610 µg/L, respectively). Cyclodiene chemi-

cals typically disrupt nervous system function by preventing chloride ions from entering neurons through inhibition of GABA receptors (Ware 1991). It has been suggested that a similar inhibition mechanism could decrease movement of the filter-feeding appendages of *D. magna*, thereby decreasing feeding efficiency (Fernandez-Casalderrey *et al.* 1994).

Endosulfan has also been shown to affect growth and reproduction in *D. magna*. Fernandez-Casalderrey *et al.* (1993) found mean carapace length was reduced from 0.79 cm in controls to 0.62 cm at a concentration of 310 µg/L. Total production of young per female decreased from 67.05 for controls to 39.56 and 24.44 at 200 µg/L and 310 µg/L endosulfan, respectively. Mean number of broods was reduced for all concentrations tested while time to reach reproductive maturity increased (Fernandez-Casalderrey *et al.* 1993).

D. magna and *P. subcapitatum* were less sensitive to endosulfan than other freshwater species tested. For example, 96-h LC₅₀ values for rainbow trout, fathead minnow, channel catfish, and bluegill sunfish range from 1.2 to 1.5 µg/L (John-

son and Finley 1980). Two aquatic invertebrates, scuds (*Gammarus lacustris*) and stoneflies (*Pteronarcys*), had 96-h LC₅₀ values of 5.8 µg/L and 3.3 µg/L, respectively (Johnson and Finley 1980). Acute toxicity of endosulfan was even greater for estuarine organisms, such as the grass shrimp *Palaemonetes pugio* (96-h LC₅₀ = 0.62 µg/L) (Wirth *et al.* 2001) and the amphipod *Gammarus palustris* (96-h LC₅₀ = 0.43 µg/L) (Leight and Van Dolah 1999). Although only moderate toxicity was observed in free-swimming *Daphnia*, endosulfan has been shown cause significant mortality to *D. pulex* embryos at concentrations as low as 0.1 µg/L (Barry 2000).

P. subcapitatum sequestered endosulfan from water, with an average BCF of 2,682. In a similar study by Rao and Lal (1987), the blue-green algae *Anabaena* sp. and *A. fertilissima* bioconcentrated endosulfan, with BCFs of 770 and 1,787, respectively after 16 h in water concentrations of 100 µg/L. They found a linear increase in bioconcentration with dose, indicating passive partitioning of the insecticide between the water and cellular lipid. Compared to these two blue-green species, we would conclude that *P. subcapitatum* has a greater uptake affinity for endosulfan.

The hypothesis that endosulfan would be trophically transferred from phytoplankton to invertebrate grazers was not supported by these experiments. *D. magna* neonates accumulated very little endosulfan when exposed to the pesticide via ingestion of contaminated phytoplankton. However, *D. magna* neonates rapidly bioaccumulated and concentrated endosulfan from contaminated water (average BCF of 3,278). Similar to our findings, concentrations of fenvalerate (a synthetic pyrethroid insecticide) in cladocerans were not increased when concentrations of fenvalerate in dosed algae, the food organism, were increased (Day and Kaushik 1987). Bahner *et al.* (1977) also did not find significant trophic transfer using the organochlorine insecticide kepone from phytoplankton to oysters. However, significant quantities of kepone were transferred from prey to predatory fish (Bahner *et al.* 1977). The BCF determined in this study for endosulfan in *D. magna* is similar to those reported for more persistent organochlorines (DDT, dieldrin, and chlordane) by daphnids (2,500–12,000) (Day 1990). The BCFs determined for phytoplankton and zooplankton in this study were higher than most BCFs reported for endosulfan in other organisms (Table 4).

Thomann *et al.* (1992) examined three exposure routes—water, sediment ingestion, and phytoplankton ingestion—by which amphipods may accumulate organic chemicals. They concluded that the predominant route of exposure for a given compound depends on the K_{ow} for that particular compound. By inserting endosulfan (log K_{ow} of approximately 3.5; ATSDR 1993), onto their graph of log K_{ow} versus factors contributing to the accumulation factor in amphipods (Figure 1), we would predict that water is the dominant route through which biotic accumulation of endosulfan would occur. For more lipophilic compounds (*e.g.*, DDT, log K_{ow} of approximately 6.19; Chiou *et al.* 1977), we would expect sediment and phytoplankton ingestion to contribute significantly to levels of pesticide found in consumer tissue (Figure 1).

A log K_{ow} of 3.5 for endosulfan suggests the concentration in the food would be 3.5 orders of magnitude greater than the concentration in the water (Nagel and Loskill 1991). We determined an endosulfan concentration of 268.2 ng/mg in the algal food compared to a water concentration of 100 µg/L (or

0.1 ng/mg) (a difference of approximately 3.42 orders of magnitude). Nagel and Loskill (1991) found that for small fish, ingestion contributes to total body burden of a compound only if the concentration in the food is five orders of magnitude higher than the concentration in the water. The bioconcentration of endosulfan observed in this study therefore is consistent with what is known about partitioning of organic compounds according to their K_{ow} .

Because *D. magna* neonates accumulated little endosulfan in the clean water–dosed algae treatment, it is possible that endosulfan contained in *P. subcapitatum* cells was not bioavailable to *D. magna* and was cleared from the gut with little or no assimilation. It is also possible that the neonates metabolized endosulfan to endosulfan sulfate, which was then excreted. The transformation of endosulfan to endosulfan sulfate has been shown to occur by biological processes (Peterson and Batley 1993). If neonates biotransformed the endosulfan they ingested into endosulfan sulfate, this would explain the higher proportion of endosulfan sulfate relative to endosulfan I and II that was detected in the clean water–dosed algae treatment water. The greater proportion of endosulfan II accumulated by *D. magna* neonates in the dosed water–dosed algae treatment compared to the dosed water–clean algae treatment, may suggest a greater uptake affinity for the beta isomer. This is contrary to what we might predict based on the log K_{ow} values of 3.55 (endosulfan I) and 3.62 (endosulfan II) (ATSDR 1993). Accumulation differences in the neonates could also be the result of algal metabolism of endosulfan. The isomer ratios detected in the *P. subcapitatum* treatment media suggest that endosulfan I was more readily converted to endosulfan sulfate than was endosulfan II. Thus far, we can only speculate on the metabolism of endosulfan by these plankton. Further research is required to determine if such biotransformation pathways exist.

The bioconcentration potential for endosulfan in *D. magna* may depend on the life stage of the organism. Liu *et al.* (1996) concluded that for the fungicide multi-effect triazole (MET) in *D. magna* the BCF was correlated closely with lipid content. As growth progressed, lipid content increased and thus increased the bioconcentration of MET in *D. magna* tissue. The lipid content of *D. magna* neonates fed a diet of *P. subcapitatum* was about 4.34%, while adults had a lipid content of approximately 20% (Liu *et al.* 1996). Based on our finding that *D. magna* neonates were able to accumulate endosulfan, adults, with their higher lipid content, should accumulate endosulfan to an even greater extent.

Lipid content may also be important in estimating pesticide bioconcentration potential in phytoplankton. Kent and Currie (1995) found that phytoplankton sensitivity to fenitrothion and bioconcentration capacity were correlated with total cellular lipid content. This relationship suggests that phytoplankton species with larger lipid fractions than the *P. subcapitatum* used in this study (*e.g.*, *Navicula* sp., 23.1% lipid versus *P. subcapitatum*, 16.7% lipid; Kent and Currie 1995) would have higher BCFs for endosulfan and other lipophilic xenobiotics.

McCall *et al.* (1988) found endosulfan concentrations ranging from 65.3–124.9 µg/L in direct runoff from test plots of tomatoes. Thus concentrations high enough to impair *D. magna* filtration and ingestion rates could occur in drainage ditches immediately adjacent to agricultural fields treated with en-

Table 4. Comparison of BCF results to other endosulfan uptake studies

| Organism | Exposure | BCF | Endosulfan Concentration used in BCF Calculation | Reference |
|------------------------------|-------------------|-------|--|-----------------------------|
| Phytoplankton | | | | |
| <i>P. subcapitatum</i> | 100 µg/L: 16 h | 2,682 | nominal | This study |
| <i>Anabaena sp.</i> | 100 µg/L: 16 h | 770 | nominal | Rao and Lal 1987 |
| <i>Aulosira fertilissima</i> | 100 µg/L: 16 h | 1,787 | nominal | Rao and Lal 1987 |
| Zooplankton | | | | |
| <i>Daphnia magna</i> | 100 µg/L: 24 h | 3,278 | nominal | This study |
| Grass shrimp | | | | |
| <i>Palaemonetes pugio</i> | 0.2 µg/L: 30 days | 200 | measured | Wirth 1999 |
| <i>Palaemonetes pugio</i> | 0.16 µg/L: 96 h | 81 | measured | Schimmel <i>et al.</i> 1977 |
| Oyster | | | | |
| <i>Crassostrea virginica</i> | 0.2 µg/L: 96 h | 330 | measured | Scott <i>et al.</i> 1990 |
| Fish | | | | |
| <i>Fundulus heteroclitus</i> | 0.2 µg/L: 96 h | 330 | measured | Scott <i>et al.</i> 1990 |
| <i>Lagodon rhomboides</i> | 0.15 µg/L: 96 h | 1,299 | measured | Schimmel <i>et al.</i> 1977 |
| <i>Leiostomus xanthurus</i> | 0.076 µg/L: 96 h | 895 | measured | Schimmel <i>et al.</i> 1977 |
| <i>Mugil cephalus</i> | 0.32 µg/L: 96 h | 1,344 | measured | Schimmel <i>et al.</i> 1977 |

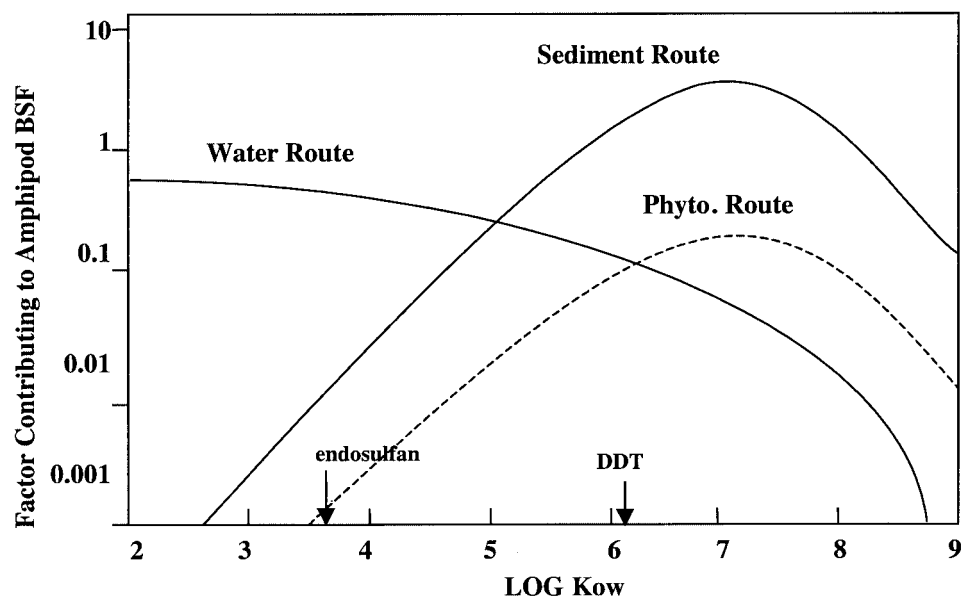


Fig. 1. Use of contaminant log K_{ow} values to predict chemical uptake based on exposure route (adapted from Thomann *et al.* 1992). Thomann *et al.* (1992) calculated the relative contribution of sediment, water, and phytoplankton routes of chemical exposure to amphipod chemical uptake. BSF (biota sediment factor) is the ratio of the organism chemical concentration to the sediment chemical concentration. Based on this model, consumption of contaminated phytoplankton would not significantly contribute to the organism BSF at the log K_{ow} of endosulfan. Water would be the dominant factor. Reprinted with permission from Thomann RV, Conolly JP, Parkerton TF (1992) An equilibrium model of organic chemical accumulation in aquatic food webs with sediment interaction. *Environ Toxicol Chem* 11:615–629. Copyright SETAC, Pensacola, Florida, USA

dosulfan. Monitoring of freshwater canals draining agricultural areas of south Florida and emptying into the Everglades and Florida Bay revealed maximum total endosulfan concentrations of approximately 0.6 µg/L (Miles and Pflueffer 1997). Though not as high as the endosulfan concentrations used in this experiment, endosulfan was detected in water samples year-round, indicating chronic exposure to aquatic organisms. Further testing should address bioconcentration effects of endosulfan in multigenerational plankton studies, using ecologically

realistic concentrations, and incorporate higher-trophic-level test organisms.

In conclusion, acute toxicity to *P. subcapitatum* and *D. magna* neonates is expected to occur at endosulfan concentrations of approximately 400 µg/L, with sublethal effects on neonate filtration and ingestion rates occurring at 166 µg/L. Endosulfan was rapidly accumulated and concentrated from water by *P. subcapitatum* and *D. magna*; however, endosulfan contained in phytoplankton was not bioaccumulated by zoo-

plankton. The results of this study may be useful in modeling the fate and effects of endosulfan in aquatic ecosystems. Based on these findings, the amount of endosulfan concentrated by phytoplankton prey species would not need to be considered in prediction of zooplankton BCF.

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