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Toxicological Studies in Tropical Ecosystems: an Ecotoxicological Risk Assessment of Pesticide Runoff in South Florida Estuarine Ecosystems

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A multiyear study in the C-111 canal system and associated sites in Florida Bay was undertaken to determine the potential pesticide risk that exists in South Florida. After the examination of extensive pesticide concentration data in surface water, tissues, and semipermeable membrane devices (SPMDs), canal contamination seems to be derived from the extensive agricultural production that drains into the C-111 canal. The results of this study indicate that runoff from agricultural processes led to quantifiable pesticide residues in both canal and bay surface water, which occasionally exceeded current water quality criteria. The major pesticide of concern was endosulfan, which was detected at 100% of the sites sampled. Endosulfan exposure did not cause any acute effects in fish and crustaceans deployed in field bioassays. Chronic effects were observed in copepods, clams, and oysters but could not be attributed to endosulfan exposure. The decision to alter the C-111 canal flow and allow increased freshwater flow into the adjacent Everglades National Park may result in discharges of pesticides into the Everglades. Continued monitoring in this area is needed during this change in flow regime.

KEYWORDS: Endosulfan; Florida Bay; South Florida; semipermeable membrane devices; oysters; copepods; clams

INTRODUCTION

In estuarine and marine areas such as Florida Bay, there have been increased phytoplankton blooms, declines in seagrass beds and coral reef communities, and a trophic shift toward plankton-feeding fish communities. These shifts have come at the expense of communities dominated by seagrass canopies and associated benthic-dwelling seagrass inhabitants (*1*). The deteriorating condition of South Florida ecosystems has led to concerns about the potential effects of chemical contaminants on ecosystem health within the region. These conditions may be related, in part, to increased nutrient and contaminant loading primarily from agriculture but also from urban and industrial discharges within the region. Additionally, shifts in faunal assemblages resulting from habitat or salinity changes have also been observed (*1*). Contaminants from urban sources may include polycyclic aromatic hydrocarbons (PAHs), trace metals (e.g., Cu), and pesticides associated with urban landscaping (e.g., chlorpyrifos), termite control (formerly chlordane and chlorpyrifos), vector (mosquito) control (e.g., organophosphates, synthetic pyrethroids, and insect growth regulators), and golf courses (including numerous insecticides, herbicides, and fungicides) (*2*, *3*). Agricultural pesticides are of great concern

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because of the large concentration of agricultural operations in close proximity to sensitive South Florida ecosystems (*4*, *5*).

Miles and Pfeuffer (*4*) reported that ~14490 tons of pesticides are applied annually in the South Florida Water Management District (SFWMD), with agricultural pesticides accounting for 84% of the total pesticide use within the region (12172 tons). In 1997, this included pesticide application on 428,000 acres of sugarcane, 213,000 acres of citrus, and 155,000 acres of vegetable crops. The pesticide usage included 38% insecticides, 20% herbicides, and 24% fungicides, nematicides, and fumigants (*4*). Agriculture in Florida contributes >$45 billion annually to the state's economy, with >$2 billion produced in the four South Florida counties of St. Lucie, Palm Beach, Hendry, and Dade (*6*). Insecticide application in South Florida is nearly double the national average, reflecting the widespread usage of insecticides to deal with the large numbers of insects (termites, mosquitoes, fire ants, and other pests) associated with this subtropical region of the United States. The most widely used insecticides include methomyl (vegetables), ethoprop and phorate (sugarcane), and chlorpyrifos (golf courses and suburban use).

Generally, vegetable farming, which is prevalent in South Florida, requires high-intensity pesticide application (>9.1 kg of all pesticide active ingredients per acre per crop). Often, there may be more than one crop per year, thereby increasing the annual amount of pesticide used. Agricultural pesticides of concern include chlorpyrifos, chlorothalonil, atrazine, and endosulfan because of their widespread use and/or high toxicity potential. Miles and Pfeuffer (*4*) detected pesticides in 2% of all surface waters sampled in canals within the SFWMD and, more notably, the insecticide endosulfan was detected in 5% of all surface-water samples. Often concentrations exceeded the U.S. EPA's chronic water quality criteria (WQC) for fresh (56 ng/L) and marine (8.7 ng/L) waters (*4*). Other studies (*7*, *8*) have also reported endosulfan in canal and surface waters of Florida Bay, often exceeding the WQC.

Water *quantity* rather than water *quality* issues have dominated planning in the South Florida restoration process. Restored water flow in the Everglades and South Florida may increase runoff of contaminants, particularly pesticides, to the southwestern coast and other regions of the state. Such concerns were detailed in the recent Workshop on Ecological Risk of Toxic Substances in South Florida Ecosystems sponsored by the South Florida Ecosystem Restoration Task Force. Workshop participants recommended screening-level risk assessments with subsequent retrospective and prospective diagnostic studies (*9*). Particular emphasis was placed on the contemporary-use pesticide endosulfan because of the high usage in the Dade County agricultural areas (*4*).

Endosulfan has caused more coastal fish kills than all other pesticides combined (*10*), and >76% of the endosulfan used in the southeastern United States discharges primarily into the Florida Bay watershed (*11*). Thus, the overall objective of this study was to determine the potential impacts of agricultural and urban pesticide discharges on natural resources of freshwater canals and estuarine ecosystems of Florida Bay, emphasizing the agricultural pesticide endosulfan.

MATERIALS AND METHODS

Field Monitoring Studies. Most of the land surface runoff from South Florida urban and agricultural areas is discharged into a series of drainage canals extending >2900 km (*6*). Water flow in these canals is highly controlled by the SFWMD. The SFWMD plans to alter the canal flow to allow more fresh water to flow into the Everglades (*6*). Concerns over this modification, particularly into the Everglades, have resulted in several research projects including this study.

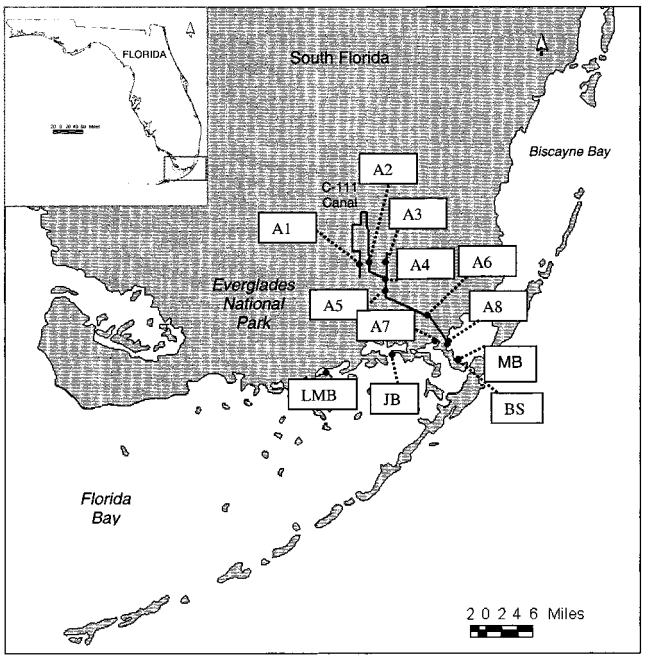


Figure 1. Map of study site.

Table 1. Detailed Study Sites along with Location and Parameters Measured at Each Site

	location			
station	latitude (°N)	longitude (°W)	habitat	parameters measured[a]
A1	25.399	80.573	canal	SW, WQ
A2	25.402	80.558	canal	SW, WQ
A3	25.3917	80.52589	canal	SW, WQ, ISB, STT, FR, SPMD
A4	25.36	80.525	canal	SW, WQ
A5	25.331	80.525	canal	SW, WQ
A6	25.29	80.455	canal	SW, WQ, ISB, FR, SPMD
A7	25.24587	80.4347	east bay	SW, WQ, ISB, STT, OR, SPMD
A8	25.26177	80.42489	east bay	SW, WQ, ISB, STT, OR, SPMD
MB	25.24967	80.39268	east bay	SW, WQ, STT
LMB	25.18799	80.63457	east bay	SW, WQ, STT
BS	25.21171	80.39260	east bay	SW, WQ, STT
JB	25.22128	80.51376	east bay	SW, WQ, STT, OR, SPMD

[a] SW, surface-water chemistry; WQ, water quality; STT, sediment toxicity tests; ISB, in situ bioassay; FR, fish residue; OR, oyster residue; SPMD, semipermeable membrane device.

Most of the water discharged into the Everglades and Florida Bay as a result of these hydrological modifications originated as runoff from urban and agricultural areas of South Florida and may contain potentially toxic compounds (see ref *4*). Field studies were conducted from February 1993 through February 1997 to assess the impacts of chemical contaminants on both canal and adjoining estuarine and marine ecosystems.

The design of field studies was to collect surface-water samples from a gradient of sites (**Figure 1**) that included freshwater stations adjacent to agricultural areas near Homestead, FL, that drain into the C-111 canal (stations A1−A6) and estuarine stations where the C-111 canal discharges into eastern Florida Bay (stations A7, A8, MB, BS, JB, and LMB). The sample location and specific parameters measured at each station are listed in **Table 1**. Sampling was generally timed to coincide with agricultural production and included sampling during both growing (high pesticide usage) and nongrowing (low pesticide usage) seasons.

Chemical Analysis. *Surface Waters.* At each station, surface-water samples (500 mL) were collected (1993−1995) and analyzed for chlorpyrifos and endosulfan (α-endosulfan, β-endosulfan, and endosul-

Table 2. Limits of Detection for Pesticides Measured in Surface Water during the Two Periods of This Study in the C-111 Canal System

pesticide	limit of detection (ng/L) 1993–1995	1996–1997
atrazine	1.0	1.0
chlorpyrifos	0.1	0.30
chlorothalonil		0.40
α-endosulfan	10	0.02
β-endosulfan	10	0.02
endosulfan sulfate	10	0.02

fan sulfate) using methods described by Scott et al. (*5*). During 1996 and 1997, water samples (5−10 L) were collected daily, at prescribed sampling intervals and following significant rainfall events (>1.27 cm/24 h), and analyzed for pesticide residues. The change in sampling protocol allowed each sample to be analyzed for a wider suite of pesticides and decreased limits of detection (**Table 2**). Samples were immediately transported to the laboratory and extracted. All samples were thoroughly shaken by hand and extracted through either C-18 (Jones Chromatography, Lakewood, CO) (1993−1995) or ENV+ (International Sorbent Technologies, Glamorgan, U.K.) (1996−1997) solid phase cartridges using methods described by Strozier (*12*) or Downing et al. (*13*). Samples were then eluted from the cartridge (C-18 with ethyl acetate and ENV+ with acetone/dichloromethane; followed by hexane). The samples were concentrated on a Turbovap (Zymark, Hopkinton, MA) at 40 °C and then solvent exchanged into >5 mL of hexane and reduced to a final volume of 0.5 mL. Samples were additionally processed by passing the extracts through Florisil to remove interfering biogenic compounds (*14*). The concentrated sample was added to the micro-Florisil column and eluted with 7 mL of a 20% (by volume) ethyl acetate in hexane solution (*13*). Each sample was then evaporated under a stream of dry nitrogen gas (ultrahigh purity = 99.999%) to near dryness and diluted to 0.5 mL with hexane.

For samples collected between 1993 and 1996, each sample was analyzed using a Hewlett-Packard GC (model 5890A, Palo Alto, CA) with an electron capture detector and a 30 m DB-5 capillary column (J&W Scientific, Folsom, CA) with a 0.25 mm diameter and a 0.25 μm film thickness. Samples were injected in the splitless mode. Chromatographic conditions are described in Strozier (*12*). Peak heights and retention times from each sample were compared to internal and external analytical standards for each compound's identification and quantification. All samples (1996−1997) were analyzed by capillary column gas chromatography (GC) and mass spectrometry as described by Harmon-Fetcho et al. (*15*). Limits of detection for samples from 1993 through 1995 were on the order of 10 ng/L for endosulfan and chlorpyrifos. Lower limits of detection decreased significantly when the GC-MS methods were used during 1996 and 1997 (**Table 2**).

Sediments and Tissues. Sediments and oyster and fish tissues were collected in 1996 (oyster and sediment) and 1996−1997 (fish) from selected field sites (A3, A7, A8, JB, and LMB for sediments; A3, A6, A7, and A8 for fish tissue) and extracted for pesticide residues using the methods of Kucklick et al. (*16*) and Scott et al. (*5*). Oysters were shucked with solvent-rinsed oyster knives in the field, and the tissues were transferred into precleaned glass jars. The oyster tissues were then immediately frozen. Fish samples were placed on ice and transported to the laboratory frozen. The fish tissue was thawed and then filleted for muscle tissue extraction. Analysis and quantification were performed using GC methods described above. Results are reported on a dry mass basis. Tissues analyzed included oysters *Crassostrea rhizophorae* (endemic) and *Crassostrea virginica* (deployed). Lipids were determined gravimetrically by extracting up to 5 g of homogenized tissue in methylene chloride and evaporating the solvent from a tared weigh pan. Tissue endosulfan concentrations were normalized to percent lipid and reported as nanograms per gram of lipid. The lipid-normalized tissue concentration was then divided by the octanol/water partition coefficient (K_{ow}; liters per kilogram) for endosulfan in order to calculate the estimated surface-water endosulfan concentrations. These estimates were compared to both water quality criteria for endosulfan and the measured surface-water concentrations at each site (following ref *17*). The lipid-based concentration was converted to nanograms per kilogram of lipid and divided by the K_{ow} for each endosulfan isomer (K_{ow} for each isomer is α = 3548, β = 4169, and sulfate = 4571; *18*), which resulted in an estimated water concentration in nanograms per liter.

$$\text{BCF (L/kg)} = \text{organism (ng/kg of lipid)/water (ng/L)} \quad \text{(a)}$$

$$K_{ow}\text{ (g of octanol/g of water)} \approx \text{BCF} \quad \text{(b)}$$

so for α-endosulfan, β-endosulfan, and endosulfan sulfate

$$\text{ng of pesticide/L of water} = [\text{ng/g of lipid}/K_{ow}\text{ (g of octanol/g of water)}] \times 1000\text{ mL/L} \quad \text{(c)}$$

The calculated water concentrations for the three endosulfan isomers (α, β, and sulfate) were then summed. Limits of detection in oysters were <0.05 ng/g of wet weight for each endosulfan isomer or endosulfan sulfate. Endosulfan sulfate was included because this metabolite has been shown to be the major environmental metabolite of concern (*19*). However, the State of Florida and the U.S. EPA quantify water quality criteria based solely upon the α and β isomers.

Fish were caught at various stations in the C-111 canal system and analyzed for pesticide residues. Species caught included *Lepomis macrochirus* (bluegill), *Lepomis punctatus* (spotted sunfish), *Cichlasoma uropthalmus* (Mayan cichlid), *Tilapia* sp. (tilapia), and *Micropterus salmoides* (largemouth bass). Fillets (with skin) were removed from the fish, ground, and extracted in a Soxhlet extraction manifold in methylene chloride. The extract was passed through an affinity column that isolated the pesticide-containing fraction (*16*). This fraction was solvent exchanged into hexane under N_2 using a Turbovap and analyzed for pesticides as above. Each fish fillet was analyzed for the following pesticides (LLODs in ng/g of dry weight): 2,4′-DDD (<0.061); 2,4′-DDE (<0.058); 2,4′-DDT (<0.144); 4,4′-DDD (<0.243); 4,4′-DDE (<0.243); 4,4′-DDT (<0.016); aldrin (<0.013); dieldrin (<0.1); chlorpyrifos (<0.082); endosulfan (<0.1 for endosulfan isomers α- and β- as well as endosulfan sulfate); heptachlor (<0.04); hexachlorobenzene (<0.062); and *trans*-nonachlor (<0.094).

In Situ Toxicity Tests. *Fish and Shrimp.* In situ, acute (96 h) toxicity tests were conducted in February 1996 at selected freshwater (A3 and A6) and marine (A7 and A8) sites using adult mosquito fish (*Gambusia holbrooki*) and grass shrimp (*Palaemonetes pugio*). At each site, organisms were deployed using methods described by Scott et al. (*5*) and Fulton et al. (*20*). Briefly, fish and shrimp were deployed in acrylic cages (n = 10/cage) covered with nylon mesh and placed in an additional exclusion cage to prevent predation. A total of three fish and three shrimp cages were deployed at each site and monitored daily for mortality and survival over 96 h.

DataSondes (Hydrolab, Austin, TX) were deployed at each of these sites to provide a continuous record of physicochemical water quality at each site. Water quality parameters included water temperature (°C), salinity (ppt), conductivity (μmhos/cm), dissolved oxygen (mg/L), pH, and water depth (m).

Oysters. Adult deployed oysters (*C. virginica*, >7.82 cm) were collected from two sources: in 1995 from Leadenwah Creek, SC, and in 1996 from Del Ray Beach, FL, and deployed at selected field sites (A7 and A8) in plastic cages. After 60 days of field exposure, oysters were monitored for survival, allometric growth indices (condition and gonadal indices), and uptake of chemical contaminants using methods described by Scott (*21*) and Scott et al. (*17*).

Sediment Toxicity Tests. Sediments from selected field sites (A3, A8, MB, BS, JB, and LMB), a Florida Bay control site (A7), and a laboratory reference sediment (North Inlet NERR, Georgetown, SC) were assessed for toxicity using juvenile clams (*Mercenaria mercenaria*) and copepods (*Amphiascus tenuiremis*) using methods described by Chung (*22*) and Chandler (*23*−*25*), respectively.

Clams (212−350 μm) were obtained from the Sea Perfect clam farm on James Island, SC, and transported to the laboratory. Sea water used for acclimation and exposure was collected from Bohicket Creek, a tidal tributary of the North Edisto River estuary in South Carolina and filtered to <20 μm. Juvenile clams were acclimated for 24−48 h in 16

oz precleaned glass jars at 20 °C and 30 ppt salinity with a 12 h light/12 h dark cycle. Clams were fed *Isochrysis galbana* from laboratory cultures prior to testing.

Sediment from each site was press-sieved through a 212 μm mesh screen. Each testing chamber consisted of a 600 mL Pyrex glass beaker, 100 mL of sieved sediment, and 300 mL of 20 μm filtered sea water at 30 ppt. For each site or control, five replicates of sediment with 50 clams in each were tested for a total of 10 days with light aeration. Toxicity tests were run at 20 °C with a 12 h light/12 h dark cycle. Clams in each beaker were fed 5 mL of *I. galbana* every 48 h. Each day, temperature, dissolved oxygen, salinity, and pH were recorded from each control replicate. On days 0, 2, and 8, total ammonia (as nitrogen) was measured. At the end of 10 days, sediments were carefully sieved and clam mortality was determined. Mortality was defined as individuals with gaping shells or lack of response to physical stimulus.

A benthic copepod (*Amphiascus tenuiremis*) life cycle assay has been developed that allows for multiple generational testing ($f_1 \rightarrow f_2$ generational testing) within a 14-day period and includes survival, reproduction, and development endpoints (*23*−*25*). For the copepod toxicity tests, sediment from each site was processed according to the method of Chandler and Green (*23*), and 10 mL aliquots of the sediment slurry from each site were placed in each of five replicate test chambers containing 20 mL of filtered artificial sea water and 25 *A. tenuiremis* adult males and 25 nongravid females. Chambers were placed in an incubator at 20 °C and 30 ppt salinity with a 12 h light/12 h dark cycle for 10 days. Water quality parameters (salinity, temperature, dissolved oxygen, pH, and ammonia) were monitored at the beginning and end of each experiment.

Upon completion of an experiment, test chambers were removed from the incubator. Contents of the chamber were washed onto a 63 μm sieve with filtered artificial sea water and rinsed into a plastic Petri dish. The contents of each dish were stained with Rose Bengal and preserved with formalin (5% of the final volume). The dishes were refrigerated until their contents were counted under a stereo dissection microscope.

Copepod toxicity test endpoints included adult female and male mortality (f_1) as well as the reproductive endpoints of the numbers of copepodite, nauplii, and clutch size (f_2). Potential (eggs + nauplii + copepodites) and realized (nauplii + copepodites) reproductions were also determined. When mortality was minimal, reproductive endpoints were compared in a general linear model (GLM) procedure using SAS (*26*) statistical software. Significant differences among sites and controls were detected using Tukey's Studentized *t* test and Dunnett's test.

Semipermeable Membrane Devices (SPMDs). SPMDs were deployed in stainless steel cages at selected field sites (A1, A3, and A6). After 96 h of exposure, SPMDs were collected, placed in solvent-cleaned sealed containers, and transported on ice to the laboratory. SPMDs were then placed in a freezer and stored at −30 °C until analyzed. For chemical analysis, each SPMD was thawed, extracted using methods in Huckins et al. (*27*), and quantified using methods described by Strozier (*12*). Results are reported in nanograms per gram of lipid. SPMD endosulfan concentrations were also divided by the K_{ow} for endosulfan in order to estimate the surface-water concentration of endosulfan and to compare these values to water quality criteria for endosulfan and the GC-measured surface-water concentrations at each site. This approach assumes that equilibrium was established after 96 h of deployment.

RESULTS

Surface Water Monitoring of Endosulfan and Other Pesticides. Results of the field studies conducted during 1993−1997 (**Tables 3** and **4**; **Figure 2**) indicated the presence of endosulfan and other pesticides in surface waters from Florida Bay and surrounding environments. Initial sampling during 1993, which had higher LLODs, also indicated the presence of endosulfan both in the canals adjacent to the agricultural areas and in Florida Bay waters. In 1994, detectable levels of endosulfan were measured ranging from 17 to 155 ng/L, but only in canal surface waters adjacent to agricultural areas.

Table 3. Average Endosulfan Concentrations Based on Site and Year and the Percent of Samples That Exceeded the Appropriate WQC (0.056 μg/L Fresh Water and 0.0087 μg/L Marine Chronic Value) at Canal and Florida Bay Sites

site	endosulfan concn (ng/L)	% of samples > WQC	no. of samples
A1	4	11.1	15
A2	9	0	14
A3	124	40.0	30
A4	6	0	15
A5	3	0	14
A6	3	0	19
A7	1	0	12
A8	1	0	20
bay sites	9	1.9	20

year	av (± SD)	peak endosulfan concn (ng/L)	no. of samples with detectable endosulfan/total samples
1993	99 (–)	99	1/9
1994	30 (±60)	155	3/9
1995	9 (±30)	287	22/85
1996	14 (±50)	205	17/80
1997	41 (±4)	301	58/70

Table 4. Frequency and Peak Concentrations of Pesticides Measured in Surface Waters of Florida Bay and the C-111 Canal (1996–1997) Using the More Sensitive Analytical Methods

pesticide	% occurrence	peak concn (ng/L)
atrazine	92.3	48.0
chlorpyrifos	80.8	2.42
chlorothalonil	84.6	2.70
total endosulfan	100	477

However, during 1995, detectable endosulfan concentrations were measured at both bay and agricultural sites. In Florida Bay, 39% of the sites had detectable endosulfan concentrations and samples ranged from LLOD to 9 ng/L. Approximately 2% of the bay sites sampled had endosulfan concentrations exceeding the chronic marine water quality criterion (8.7 ng/L) for endosulfan (*28*) (**Table 3**). Water from canal sites adjacent to agricultural areas had detectable levels of endosulfan in 47% of the samples. Approximately 10% of the samples from the canal sites had endosulfan concentrations exceeding the chronic freshwater water quality criterion (56 ng/L) for endosulfan (**Table 3**).

In February 1996, detectable levels of endosulfan were measured in surface waters at all sites sampled during a sampling period associated with the winter vegetable farming season (**Table 4**). All bay sites had endosulfan concentrations ranging from 0.3 to 2.3 ng/L. Similarly, all of the agricultural sites had detectable concentrations of endosulfan, ranging from 1.7 to 205 ng/L. Only one sample (from site A3) contained endosulfan residues above the U.S. EPA water quality criteria for fresh water. There were no marine WQC violations.

As a result of the lower detection limits achieved during the 1997 surface-water monitoring, 100% (nine of nine) of the sites sampled had detectable levels of endosulfan from the winter (February) sampling trip, and seven of nine sites had detectable levels during the fall (September) sampling. Concentrations reached 292 ng/L at the canal outfall (A8) and 477 ng/L in the C-111 canal (A3). The highest levels measured in the canal (seven measurements) and bay (one measurement) exceeded EPA fresh and marine WQC values, respectively (**Table 3**).

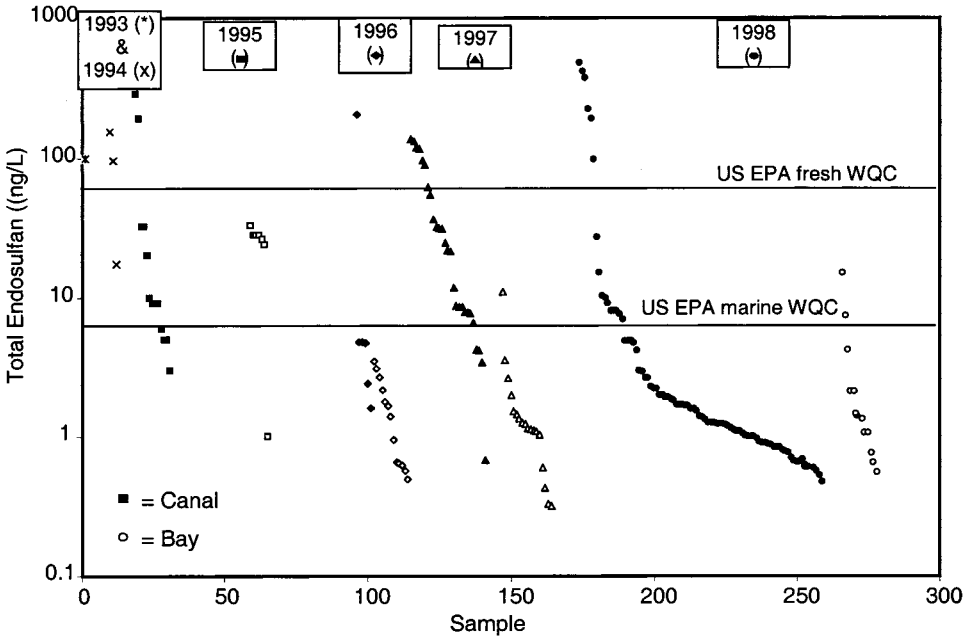


Figure 2. Endosulfan concentrations in canals and surface waters of Florida Bay.

Table 5. Endosulfan Residues Quantified from Deployed Oysters and SPMDs

oysters	site	total endosulfan (ng/g of wet wt)	lipid normalized (ng/g of lipid)	estimated water concn (ng/L)	actual water concn (ng/L)
C. virginica	initial	<0.5	<0.5		
C. virginica	A7	<0.5	<0.5		
C. virginica	A8	3.2	320	76.9	3.5
C. rhizophorae	Joe Bay	<0.5	<0.5		
C. rhizophorae	LMB	2.1	102	24.6	1.7
C. rhizophorae	A8	1.6	119	30.8	3.5

	site	bag concn (ng/g of lipid)	estimated water concn (ng/L)	actual water concn (ng/L)
SPMD	A1	<0.5		2
	A1	<0.5		2
	A1	<0.5		2
	A3	464	123.8	299
	A3	485	130.3	299
	A3	364	96.9	299
	A6	<0.5		1
	A6	<0.5		1
	A6	<0.5		1

Results from 1998 indicated much higher endosulfan concentrations (>400 ng/L) in canals sites, but bay concentrations measured were not significantly elevated. There was a subsequent drop in canal site concentrations in the August 1998 (nonagricultural growing season) sampling period. In addition to endosulfan, other pesticides measured with a high frequency in Florida Bay during 1996 and 1997 included atrazine (92% of sites sampled), chlorothalonil (85%), and chlorpyrifos (81%) (**Table 4**).

SPMD. SPMDs were deployed in 1998 at three sites (A1, A3, and A6). Measured water concentrations ranged from <LLOD to 485 ng/g of lipid. Calculated endosulfan values were 437 ng/L (±65 ng/L) at site A3 and <LLOD at A1 and A6 (**Table 5**). Endosulfan concentrations reached a maximum of 485 ng/g of lipid (or estimated to be 130 ng/L following the previous equations) at the canal site (A3). During the fall sampling trip, estimated endosulfan levels in the water at site A3 (as calculated previously), based on the SPMD results, averaged 299 ng/ L (±108 ng/L).

Sediment and Tissue Analysis. Sediments from the C-111 canal and Florida Bay were collected in 1996 from five sites (A3, A7, A8, JB, and LMB) and analyzed for total endosulfan and chlorpyrifos. Chlorpyrifos did not exceed the LLOD of 0.1 ng/g of dry weight from any sample. Total endosulfan values were 168 and 1.33 ng/g of dry weight for A3 and JB, respectively. Samples from other sites did not contain endosulfan residues >LLOD.

Fish fillets were analyzed for a variety of pesticides. Of these compounds, the following were <LLOD: 2,4′-DDD; 2,4′-DDE; 2,4′-DDT; 4,4′-DDD; 4,4′-DDT; aldrin; dieldrin; and chlorpyrifos. Species with measurable levels of pesticides included bluegill, Mayan cichlid, tilapia, spotted sunfish, and largemouth bass (**Figure 3**).

Indigenous mangrove oysters (*C. rhizophorae*) were analyzed for endosulfan at selected sites. Oysters (*C. virginica*) from South Carolina were deployed for 60 days at selected sites and also analyzed for endosulfan. Results obtained from the tissue analysis (**Table 5**) were then used to estimate water concentrations by first calculating the endosulfan concentration on a nanograms of endosulfan per gram of lipid basis. Endosulfan concentrations from *C. rhizophorae* ranged from <LLOD to 2.05 ng/g of wet weight (<LLOD−119.2 ng/g of lipid). Additionally, endosulfan concentrations measured in *C. virginica* ranged from <LLOD to 3.21 ng/g of wet weight (<LLOD−319 ng/g of lipid). Estimated endosulfan concentrations in water reached a maximum of 76.9 ng/L (**Table 5**).

In Situ Toxicity Tests. In situ toxicity tests were conducted in freshwater regions of the C-111 canal (A3 and A5) using the mosquito fish (*G. holbrooki*) and in marine waters (A7 and A8) using the grass shrimp (*P. pugio*). Significant mortality did not occur except during the wet season sampling of fall 1997, when 100% mortality occurred at site A3 (**Table 6**). This mortality was attributed to an unusually low dissolved oxygen level recorded during the testing period.

Sediment Toxicity Tests. Results of the sediment toxicity tests with copepods and clams (**Figure 4**) indicated that sediments from canals and eastern Florida Bay sites were toxic.

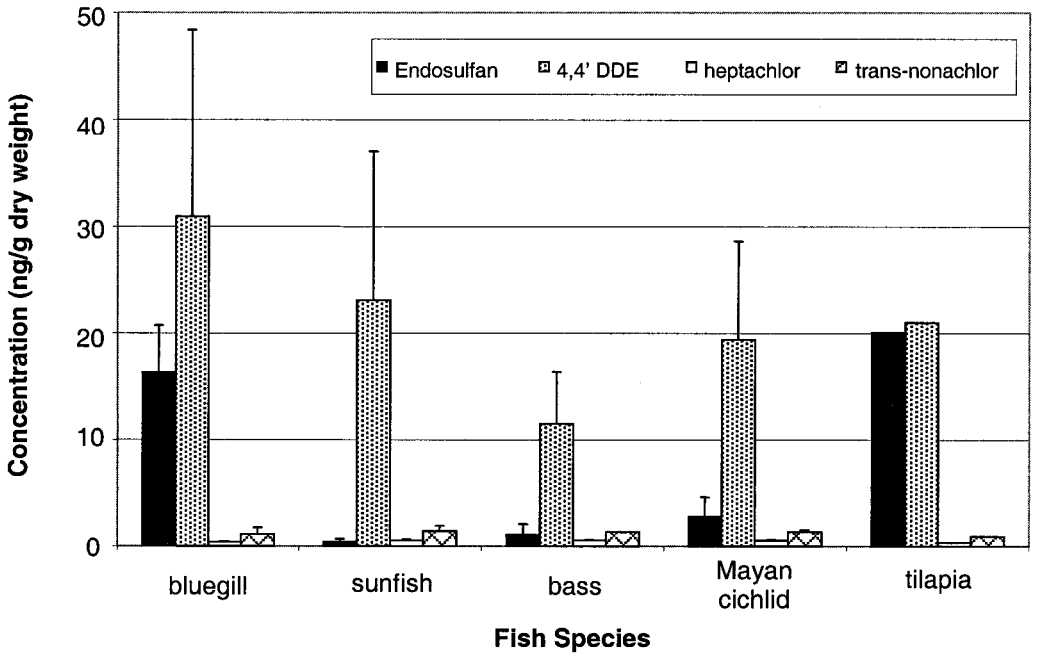


Figure 3. Freshwater fish endosulfan concentrations.

Table 6. In Situ Toxicity Tests Results for Mosquitofish and Grass Shrimp Deployed during 1996 and 1997

		% survival			
organism	site[a]	Feb 1996	Feb 1997	Sept 1997	av
G. holbrooki	A3	63.3	80	0[b]	71.7
	A6	80	83.3	63.3	75.5
	lab control		96.6	83.3	90.0
P. pugio	A7	100	100	100	100
	A8	93.3	96.6	100	96.6
	lab control		93.3	100	96.7

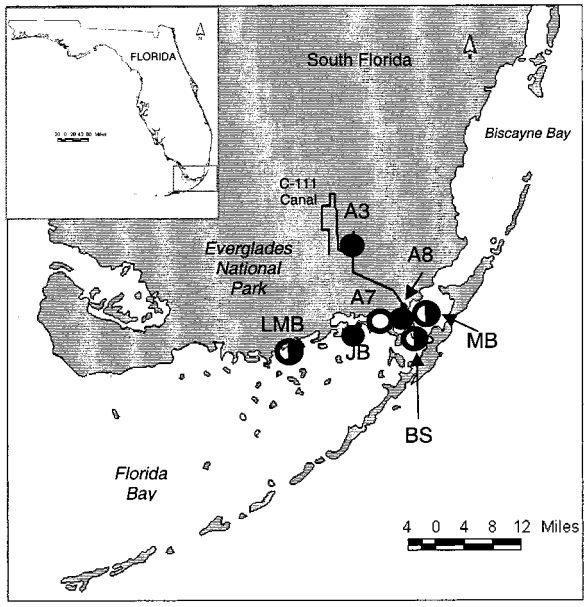
[a] Laboratory controls were not run during the 1996 deployment. [b] *G. holbrooki* samples at A3 were associated with a severe depletion of dissolved oxygen and not included in the average survival.

Copepod survival was significantly reduced ($p < 0.05$) in the percent of surviving males (selective male toxicity at JB and A8), percent of surviving females (A3, A8, JB, and LMB), and percent of surviving gravid females (A3, A8, JB, LMB, and BS).

ANOVA with multiple-comparison (Dunnett's and Tukeys) statistical analyses indicated that reproduction was significantly affected at sites closest to the C-111 canal. Endpoints affected included reduced naupliar production (A3, A8, LMB, and JB), reduced copepodite production (A3, A8, LMB, JB, and BS), and reduced clutch size per female (A8 and BS). Overall, the percentage of gravid females ranged from 69 to 82% ($\bar{x}$ =74.4%) in Florida Bay reference and control sediments versus 36−58% ($\bar{x}$ = 51%) at all Florida Bay Sites (an overall 31.5% reduction in the percent of gravid females).

Clam toxicity tests results indicated a similar pattern with high toxicity at all canal sites and JB (**Figure 4**). Toxicity was observed in both species (clam and copepod) only at sites in the C-111 canal and eastern Florida Bay. This suggests that contamination from the canal (potentially agricultural runoff) was toxic to both crustaceans and molluscs (**Figure 4**).

Oyster Toxicity Tests. Results of the condition and gonadal indices (CI and GI) of oysters deployed at the end of the C-111 Canal, a Florida Bay reference site, demonstrated significant reductions in condition and gonadal indices in oysters during



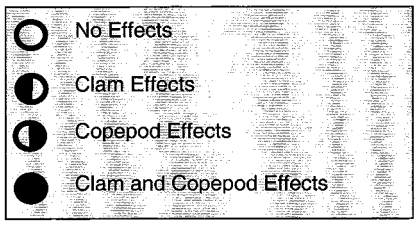


Figure 4. Sediment toxicity tests results for 1996–1997.

both 1995 and 1996 (**Figure 5**) relative to the CI and GI a the time of deployment. The CI for oysters deployed at A7 and A8 for 60 days showed a decrease between 80% (A7) and 43% (A8) of the CI at the time of deployment. Additionally, there was a 53% reduction in the CI for oysters deployed at A8 when compared to those deployed at A7. There was a marked increase in the GI of all deployed oysters. Oysters at A7 increased by >500% compared to time zero GI, and those at site A8 increased

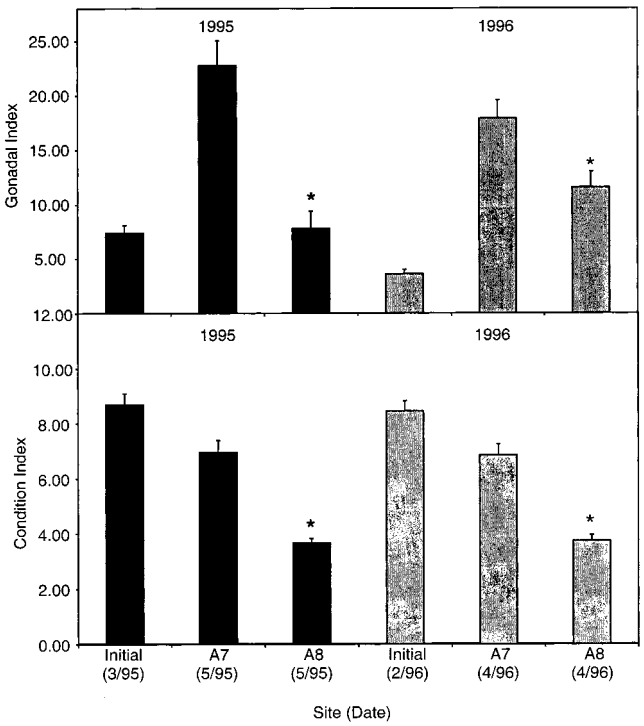


Figure 5. Oyster condition and gonadal indices for 1995–1996.

by >250%. The GI increases are likely due to the seasonal deployment time (late winter to spring) and the fact that the oysters were preparing for spawning.

DISCUSSION

Agricultural pesticides were of greatest concern during the field monitoring studies because of the large concentration of vegetable farming in close proximity to Florida Bay. Historically, agricultural pesticides of concern included azinphos-methyl, fenvalerate, and endosulfan because of their potential high toxicity to aquatic organisms (supertoxic = 96 h LC_{50} values of <10 μg/L based on U.S. Fish and Wildlife hazard ranking). However, from this list, only endosulfan is currently in use within the South Florida region. Thus, endosulfan was chosen as the primary contaminant for further toxicological studies because of the high usage on vegetable crops in South Florida (e.g., >70% of the endosulfan in the southeastern United States is applied to vegetable crops in South Florida).

From 1993 to 1998, endosulfan was primarily detected at sites A1 and A3, which are at the headwaters of the agricultural fields in this region. The data indicated that the mean α-endosulfan isomer concentration in the bay (A7, A8, BS, LMB, MB, and JB) was 1.2 ($\pm$ 0.2 ng/L) versus a mean concentration of 6.5 ng/L ($\pm$ 4 ng/L) in agricultural areas (A1−A6). Thus, α-endosulfan concentrations were 83% lower in the bay sites than in the canal at sites adjacent to agricultural areas. Similar results have been found in Texas for other pesticides (aldicarb, carbofuran, and atrazine), where water concentrations were 90% lower than surface waters adjacent to agricultural areas (*29*). Additionally, β-endosulfan was only detected in surface waters in the agricultural areas and in those bay sites adjacent to the C-111 canal (e.g., A7 and BS). These findings for the spatial distributions of β-endosulfan suggest that the source of the endosulfan was from agricultural runoff.

During 1996, sampling of surface waters was conducted with improved sampling (e.g., larger sample volumes), extraction, and analytical methodologies that allowed for lower detection limits. This improved method allowed for more precise analysis of surface waters from the Florida Bay watershed.

SPMDs. Results of the levels of endosulfan in surface waters at selected sites (A1, A3, and A6) were compared with the mean estimated surface-water endosulfan concentrations calculated from SPMDs. At site A3, adjacent to an intensive agricultural farming area, estimated surface-water endosulfan levels derived from fish (endosulfan body burden/log K_{ow}) and SPMDs yielded similar estimates. At A3, actual water concentrations averaged 124 ng/L, and the estimates of water concentrations from three SPMDs deployed at A3 ranged from 97 to 130 ng/L. The use of SPMDs seems to indicate that bioconcentration of endosulfan in biota is predictable and can be modeled using a fugacity (e.g., K_{ow}−lipid normalization) approach. Additionally, both estimated and actual endosulfan surface-water concentrations were greater than the EPA chronic freshwater quality criteria for endosulfan. Similar results were obtained at other sites including both brackish and estuarine/marine sites using SPMDs, fish, and oysters. At marine sites, oysters and SPMDs indicated that mean endosulfan surface-water concentrations were just below the EPA's WQC and were similar to surface-water concentrations measured in the field.

In Situ Toxicity Tests. Generally, there was no mortality observed during the in situ toxicity tests. However, during the fall 1997 test, nearly 100% mortality was recorded for fish at site A3. During this time, a rain event (>4 cm of rain/24 h) resulted in the displacement of low dissolved oxygenated (<0.05 mg/L) ground water into the canals. These findings have significant management implications. If low dissolved oxygen waters with potentially high biological oxygen demand are discharged into the Everglades National Park, impacts may occur in biological communities within the receiving waters. It would be prudent to maintain constant monitoring of surface-water dissolved oxygen levels in the C-111 canal prior to discharge of waters to the Everglades to ensure that high BOD demanding waters are not released into these sensitive aquatic habitats.

Oyster Toxicity Tests. The observed effects on oysters in this study are consistent with results of studies of agricultural NPS runoff in South Carolina, in which significant reductions in condition and gonadal indices were observed immediately downstream of a major vegetable farming area following significant runoff events (*30*). During this runoff event, oysters bioconcentrated endosulfan (30−113 μg/kg) and other insecticides (*30*). Roberts (*31*) found reduced spawning and condition index in mussels exposed to endosulfan. Results from this study confirmed the bioconcentration of endosulfan in deployed oysters, but more interesting is the fact that reference site oysters at A7 were exposed to lower salinities and higher endosulfan surface-water concentrations than oysters at the end of the C-111 canal. This is the result of the removal of the western levee of the canal that has allowed more freshwater flow into Long Sound. Studies conducted during the fall (e.g., wet season sampling) found that salinities were so low (<3 ppt) that 100% mortality in deployed oysters at A7 occurred.

Reductions in CI and GI are indicative of stress in oysters (*32*). Generally, as oysters are stressed due to pollutant exposures, the metabolic rate increases, resulting in energy being diverted from reproduction to maintenance metabolism (*20*, *33*, *34*). This ultimately results in reductions in oyster health. Endosulfan exposures in South Florida co-occurred with reduced salinities that generally accompany pesticide runoff (*5*), and Marcus (*30*) reported that South Carolina oysters responded to reduced salinities and pesticide contamination with reduced CI and GI as well.

Fish Analyses. In our study, a total of 20 fish were caught in the C-111 canal system. The average concentration of total endosulfan in these fish ranged from 0.3 to 20.1 ng/g of dry weight, depending on the location and species of fish. Whereas these fish fillets displayed a wide range of endosulfan concentrations, another South Florida study (*8*) found no detectable pesticide concentrations (including endosulfan) in fillets of six fish samples, one of which was from the C-111 canal and the others were from northeastern Florida Bay. Analysis of carnivorous fish (bass and Mayan cichlids) showed lower endosulfan levels when compared to the herbivorous (tilapia) or omnivorous (bluegill and spotted sunfish) fish. The sample size for each species is relatively small and, in this study, any quantitative discussion is premature. However, recent data on unfed oysters and fish exposed to aqueous endosulfan had similar tissue residues, suggesting that uptake is not associated with diet (P. Pennington, University of South Carolina, personal communication). This suggestion agrees with recent models in which gill uptake was suggested as the main source of bioaccumulation in pesticides with log K_{ow} values of <5.0 (*35*).

Sediment Toxicity Tests. Juvenile clam and copepods were exposed to sediments from the Florida Bay watershed. In copepods, the percent of gravid females ranged from 69 to 82% (average = 74%) in Florida Bay reference and control sediments versus 36−58% (average = 51%) at all other Florida Bay sites. This represents an overall 31.5% reduction in the percent of gravid females. Although this difference for all pooled Florida Bay sites was not significant ($p < 0.09$), the reduced clutch size and reduced naupliar production suggest potential alterations in reproduction and development in copepods in certain regions of Florida Bay closest to the C-111 canal. Of additional interest was the finding of selective male toxicity at certain Florida Bay sites (Joe Bay and the end of the C-111 canal).

Clam toxicity test results indicated a similar pattern, with high toxicity at canal sites and JB. It is important to note that toxicity was observed in both species at sites near the C-111 canal, suggesting that runoff from the canal was toxic to crustaceans and molluscs. Although there was no apparent relationship between sediment contamination related to endosulfan and benthic organism toxicity, Scott et al. (*7*) have also reported various PAH and metal contaminations within Florida Bay and the C-111 canal. These results, in conjunction with the pesticide assessment reported in this study, may help to explain the need for continued monitoring and assessment in this area to better define sources of toxicity within Florida Bay.

In summary, the results of our study in the C-111 canal and adjacent bay sites from 1993 to 1998 are as follows:

(1) EPA chronic freshwater quality criteria (56 ng/L) for endosulfan were exceeded during each agricultural growing season from 1993 to 1998.

(2) EPA chronic saltwater quality criteria (8.7 ng/L) for endosulfan were also exceeded during 1995 and 1997.

(3) Oysters and fish accumulated endosulfan at concentrations between 3 ng/g of wet weight (*C. virginica*) and 20 ng/g of dry weight (tilapia), indicating that the endosulfan contamination source and any potential effects may be realized locally.

(4) Field and laboratory bioassay results indicated endosulfan did not cause acute toxicity; however, chronic effects related to reproduction and chronic toxicity was observed in both bivalves and crustaceans.

In terms of the current project to alter water flow into the Everglades National Park, the potential for agriculturally associated pesticide contamination appears to be relatively high, and efforts to continue to increase freshwater flow into the Everglades from the C-111 canal system should involve continued monitoring.

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