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Endosulfan and its metabolite, endosulfan sulfate, in freshwater ecosystems of South Florida: a probabilistic aquatic ecological risk assessment

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Abstract Endosulfan is an insecticide–acaricide used in South Florida and is one of the remaining organochlorine insecticides registered under the Federal Insecticide Fungicide and Rodenticide Act by the U.S.EPA. The technical grade material consists of two isomers (α-, β-) and the main environmental metabolite in water, sediment and tissue is endosulfan sulfate through oxidation. A comprehensive probabilistic aquatic ecological risk assessment was conducted to determine the potential risks of existing exposures to endosulfan and endosulfan sulfate in freshwaters of South Florida based on historical data (1992–2007). The assessment included hazard assessment (Tier 1) followed by probabilistic risk assessment (Tier 2). Tier 1 compared actual measured concentrations in surface freshwaters of 47 sites in South Florida from historical data to U.S.EPA numerical water quality criteria. Based on results of Tier 1, Tier 2 focused on the acute and chronic risks of endosulfan at nine sites by comparing distributions of surface water exposure concentrations of endosulfan [i.e., for total endosulfan (summation of concentrations of α- and β-isomers plus the sulfate), α- plus β-endosulfan, and endosulfan sulfate (alone)] with distributions of species effects from laboratory toxicity data. In Tier 2 the distribution of total endosulfan in fish tissue (whole body) from South Florida freshwaters was also used to determine the probability of exceeding a distribution of whole body residues of endosulfan producing mortality (critical lethal residues). Tier 1 showed the majority of endosulfan water quality violations in South Florida were at locations S-178 followed by S-177 in the C-111 system (southeastern boundary of Everglades National Park (ENP)). Nine surface water sampling sites were chosen for Tier 2. Tier 2 showed the highest potentially affected fraction of toxicity values (>10%) by the estimated 90th centile exposure concentration (total endosulfan) was at S-178. At all other freshwater sites there were <5% of the toxicity values exceeded. Potential chronic risk (9.2% for total endosulfan) was only found at S-178 and all other sites were <5%. Joint probability curves showed the higher probability of risk at S-178 than at S-177. The freshwater fish species which contain tissue concentrations of endosulfan (total) with the highest potential risk for lethal whole body tissue residues were marsh killifish, flagfish and mosquitofish. Based on existing surface water exposures and available aquatic toxicity data, there are potential risks of total endosulfan to freshwater organisms in South Florida. Although there are uncertainties, the presence of tissue concentrations of endosulfan in small demersal fish, is of ecological significance since these fish support higher trophic level species, such as wading birds.

Keywords Comprehensive Everglades Restoration Plan (CERP) · Endosulfan · α-endosulfan · β-endosulfan · Endosulfan sulfate · Everglades National Park · Organochlorines · Probabilistic aquatic ecological risk assessment · South Florida · Internal effect concentration · Criticial body residue · Lethal body burden · Species sensitivity distribution

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Introduction

Endosulfan is an organochlorine insecticide–acaricide, which was introduced in the mid-1950s by Hoechst AG (now Bayer CropScience) for use on a wide variety of row crops, fruits, nuts, vegetables, cotton and tobacco to control a broad-spectrum of insect pests and mites (Goebel et al. 1982). It is one of the remaining organochlorine pesticides registered under the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) by the U.S.EPA. It is a sulfur-bearing polychlorinated cyclodiene and the technically active parent compound is a diastereomeric mixture of two biologically active isomers; 70% α (alpha)- and 30% β (beta)-endosulfan. Endosulfan is commercially marketed under the registered trade name Thiodan®.

After application, endosulfan can adsorb to particulates and persist in soil and/or sediment or dissipate as a result of volatility and drift to locations far removed from the initial site of use (NRCC 1975; GFEA 2007). The main transformation product through oxidation in freshwater and saltwater, including sediment, is endosulfan sulfate (Navarro et al. 2000; Shivaramaiah et al. 2005), although the diol, α-hydroxy-ether, ether and lactone have also been detected (NRCC 1975).

Endosulfan is also one of the most ubiquitous organochlorine insecticides in the atmosphere (Shen et al. 2005). The recent nationwide pesticides air surveillance program in Canada (Canadian Atmospheric Network for Currently Used Pesticides or CANCUP) for 2004–2005 indicated that endosulfan (α- and β-isomers) was the most frequently detected organochlorine compound across the country with high wet deposition fluxes, suggesting important atmospheric inputs (Yao et al. 2008). It is therefore not surprising that both α- and β-isomers and endosulfan sulfate have been detected in surface fresh- and salt-waters, sediment and biota throughout the world, including the Arctic regions (GFEA 2007; U.S.EPA 2007).

The National Research Council of Canada (NRCC) environmental quality review of endosulfan in the early 1970s documented that the β-isomer and the principal degradation product endosulfan sulfate are highly persistent, especially in sediment, and that aquatic organisms adjacent to application sites will be exposed to "short-term peak concentrations rather than a long-term, chronic exposure" and "the exception to this pattern may be soil- and sediment-dwelling organisms" which "may be exposed to a chronic dose as well as a short-term dose" (NRCC 1975). The review states that fish are "highly sensitive to endosulfan and the sulfate" and that environmental "levels of endosulfan and the sulfate should receive particular attention".

Over the last 30 years, endosulfan has received considerable attention and it has been the subject of a number of international regulations and action plans worldwide (GFEA 2007). The National Oceanic and Atmospheric Administration (NOAA), in the early 1990s listed endosulfan, as a pesticide of concern since it was the most hazardous of 35 inventoried pesticides detected in U.S. coastal areas based on "its acute toxicity, high bioconcentration factor, and fairly long soil half-life" (Pait et al. 1992). In addition, a review of the U.S.EPA Ecological Incident Information System shows that, since 1971, there were 106 reported fish mortality-related incidents in the U.S. associated with the use of endosulfan (U.S.EPA 2002, 2007). Endosulfan has been proposed for global ban under the Stockholm Convention on Persistent Organic Pollutants (POPs) because it meets the criteria for persistence and potential to cause adverse effects, bioaccumulation and long-range transport (Latre and Ramos 2009). It is now banned in more than 50 countries, including the European Union and several Asian and Western nations. Bayer CropScience stated that it will stop selling all products containing endosulfan by the end of 2010 (K. Keteles, personal communication, July 27, 2009). Currently India and Israel are the largest endosulfan producers.

The U.S.EPA (2002) recent aquatic probabilistic risk assessment (PRA) for agriculture uses of endosulfan in the reregistration eligibility decision (RED) for a scenario involving tomato applications in Florida, resulted in joint probability curves (JPCs) predicting that, "in any single year there is a 90% probability that 60% of the aquatic species will be killed, a 50% probability that 75% of the species will be killed, and a 10% probability that 90% of the species will be killed" adjacent to fields treated at typical application rates. Furthermore, the most recent 2007 addendum to the U.S.EPA RED confirms concern for endosulfan (and the sulfate degradate) use in agriculture since it is "persistent and represents a source for endosulfan to enter aquatic and terrestrial food chains" (U.S.EPA 2007).

In South Florida, the α- and β-endosulfan isomers and endosulfan sulfate have been detected in surface water (fresh- and salt-water) and sediment (fresh- and salt-water) since the early 1990s in monitoring studies conducted by the South Florida Water Management District (SFWMD) (Miles and Pfeuffer 1997; Pfeuffer and Rand 2004), NOAA (Scott et al. 1994, 2002; Fulton et al. 2004), U.S.EPA (Goodman et al. 1999) and U.S. Department of Agriculture (Harman-Fetcho et al. 2005). At several fresh- and salt-water sampling sites in these monitoring studies, U.S.EPA water quality criteria (WQC) for freshwater and marine life were exceeded for endosulfan. A recent aquatic PRA for endosulfan in surface waters from 1999 to 2000 in South Florida indicated potential acute risks to fish and arthropods in fresh-and salt-water (Carriger and Rand 2008a, b). In addition, a PRA of sediment data for pesticides from

1990 to 2002 in South Florida freshwater canals identified endosulfan as a chemical of potential ecological concern, based on exceedences of sediment quality criteria or screening benchmarks (Carriger et al. 2006). Endosulfan had the highest potential chronic risk out of four legacy organochlorine pesticides present (i.e., DDT, DDD, DDE, chlordane) in sediment based on the probability of predicted pore water concentrations in canals exceeding the estimated no observed effect concentration (NOEC) 10th centile of the arthropod chronic species sensitivity distribution (SSD).

The South Florida ecosystem is a "heterogenous system of wetlands, uplands, and coastal and marine areas" which encompasses all or portions of 16 counties and includes two National Parks (Everglades and Biscayne), and Big Cypress National Preserve (Science Subgroup 1996). Furthermore, there are a large number of threatened and endangered plant and animal species and species of special concern in South Florida listed under the Federal Endangered Species Act, by the Florida Game and Fresh Water Fish Commission and by the Florida Department of Agriculture and Consumer Services (FDACS). South Florida is presently undergoing one of the largest environmental restoration efforts in the world, at an estimated cost of 19.7 billion dollars and possibly spanning 40 years (GAO 2007).

Agriculture is a significant land use in South Florida and the presence of endosulfan isomers and endosulfan sulfate, in surface waters, in this region, is a major concern to the U.S.EPA (2002, 2007). Recently, the total amount of active ingredient endosulfan used in Florida was estimated by the FDACS to be 98,302 lbs (FDACS 2003). Although monitoring data for surface water are available, there is no comprehensive aquatic risk assessment for endosulfan, its isomers, or for its major environmental metabolite, endosulfan sulfate, in freshwater ecosystems of South Florida. In general, there are a limited number of probabilistic aquatic ecological risk assessments for pesticides in South Florida (Carriger et al. 2006; Carriger and Rand 2008a, b; Schuler and Rand 2008). Therefore, the objective of this study was to conduct a comprehensive probabilistic aquatic ecological PRA to quantify the likelihood and extent that adverse effects to aquatic organisms are occurring from existing surface water exposures to total endosulfan (i.e., summation of concentrations of α- and β-isomers plus the sulfate), to α- plus β-endosulfan, and to endosulfan sulfate (alone) in South Florida freshwater ecosystems. In this study, a PRA approach was used with the U.S.EPA ecological risk assessment (ERA) framework (U.S.EPA 1998), which compares probability distributions of both actual exposure concentrations of endosulfan in surface freshwaters with species effects data from laboratory toxicity studies to determine the degree of overlap, which is a measure of risk. We also evaluated the significance of tissue concentrations of endosulfan detected in native fish species collected in these ecosystems using a critical body residue/lethal body burden approach (McCarty and Mackay 1993).

Methods

The probabilistic aquatic ecological risk assessment followed a two-tier approach which has been endorsed by ECOFRAM (1999). Within the tiers, the first three phases of the U.S.EPA ERA framework were incorporated: problem formulation, risk analysis and risk characterization (U.S.EPA 1998). Problem formulation describes the key properties of endosulfan including mode of action, expected ecological effects, ecosystem at risk, and assessment (what we are trying to protect) and measurement (tools used to measure effects on assessment endpoints) endpoints. Risk analysis examines two components: environmental exposure (i.e., surface water concentrations) and ecological effects (i.e., toxicity studies). In addition to surface water concentrations of endosulfan (α- and β-) isomers and sulfate, we also evaluated critical body residues of endosulfan in fish tissue. Exposure and effects data were integrated in the final risk characterization phase to determine the likelihood of effects. Risk characterization integrated probability distributions of exposure concentrations with SSDs and included use of joint probability curves (JPCs or exceedence profiles) for sites which displayed the highest potential acute and chronic risks (Solomon et al. 2000). Joint probability curves characterized the relationship between magnitude of effect and the probability of occurrence for that effect (ECOFRAM 1999).

In Tier 1, actual measured environmental concentrations (MECs) of endosulfan in surface freshwaters in South Florida were compared to the U.S.EPA WQC values (i.e., CMC or criteria maximum concentration; and CCC or criterion continuous concentration) to obtain a hazard quotient (HQ) (U.S.EPA 1980). Data on MECs for α-endosulfan, β-endosulfan and endosulfan sulfate from 47 sites in the 16 counties of South Florida from April 1992 to December 2007 were obtained from the SFWMD's DBHYDRO monitoring database (http://my.sfwmd.gov/pls/portal/url/page/PG_GRP_SFWMD_ERA/PG_SFWMD_ERAdbhydrobrowser). Site numbers and the location of sampling sites where concentrations were measured with major agricultural areas are shown in Fig. 1.

When the quotient of the exposure concentration to the criteria value was greater than 1, an adverse effect (i.e., high hazard) was expected to occur. For endosulfan, there are U.S.EPA WQC for α- and β-endosulfan: 0.22 µg/L

Fig. 1 South Florida Water Management District (SFWMD) sites with endosulfan monitoring data. Black circles indicate sites with exceedences of water quality criteria. White circles indicate sites with analytical data but no exceedences. The black dotted line is the Everglades National Park (ENP) boundary

(CMC) and 0.056 µg/L (CCC) but no criterion exists for endosulfan sulfate (U.S.EPA 1980). In Tier 1, we compared total endosulfan (i.e., summation of concentrations of α- and β-isomers plus endosulfan sulfate), α- plus β-endosulfan, and endosulfan sulfate (alone) concentrations at a site to the CMC and CCC from the U.S.EPA WQC to obtain an HQ. Sites with exceedences of the HQ in Tier 1 or sufficient data points for creating regressions for both exposure and effects were then used to focus on sites for Tier 2-Probabilistic Risk Assessment. Tier 2 included risk analyses and risk characterization.

Problem formulation

Key properties of endosulfan

Endosulfan has very low water solubility (60–100 µg/L range) and a moderate to intermediate volatility (vapor pressure: 10^{-5}–10^{-7}mm Hg) (ATSDR 2000). The α-isomer is more volatile and less persistent than the β-isomer, which is more volatile than endosulfan sulfate (NRCC 1975; Goebel et al. 1982; Guerin 2001). Henry's law constant (i.e., ratio of vapor pressure in air to liquid phase water solubility) of the α-isomer (6.7×10^{-6} atm m^3 mol^{-1}) is almost an order of magnitude greater than the β-isomer (6.7×10^{-7} atm m^3 mol^{-1}) indicating it is more rapidly dissipated from water. Volatilization will thus be a major removal route for endosulfan from the aquatic environment (NRCC 1975). Recently it has been shown that dissipation by volatilization from surface water is significant in the field (Laabs et al. 2007; Pablo and Hyne 2009). Enhanced rates of volatilization of the α-isomer are evidenced by higher concentrations of this isomer in the atmosphere (Yao et al. 2008).

The log of the octanol–water partition coefficient (log K_{ow}) is >3–4.8 for the two isomers and endosulfan sulfate (GFEA 2007; U.S.EPA 2002). The distribution coefficient, K_d, between soil and water of the β-isomer is higher than that of the α-isomer (Peterson and Batley 1993; U.S.EPA 2002; Zhou et al. 2003) and the β-isomer, is preferentially adsorbed compared to the α-isomer, from water to a range of surfaces, including sediments (Walse et al. 2002; Peterson and Batley 1993). The β-isomer is thus more persistent. The K_d for endosulfan also increases with increases in soil organic carbon content (Peterson and Batley 1993; Parkpian et al. 1998). The high organic carbon normalized sorption coefficients (K_{oc} = 10, 600–13,500) for endosulfan (technical) and the α- and β-isomers (U.S.EPA 2002; Wauchope et al. 1992) indicate they all strongly adsorb to soils and sediments.

Hydrolysis of both isomers is the likely degradation route in alkaline waters (pH >7) (NRCC 1975; U.S.EPA 2002). Endosulfan diol is the major decomposition product from alkaline hydrolysis (Goebel et al. 1982). Under acidic conditions (pH <7), both isomers are stable to hydrolysis. However, the β-isomer is more readily hydrolyzed to endosulfan diol in water (Cotham and Bidleman 1989; Miles and Moy 1979; Peterson and Batley 1993; Walse et al. 2002, 2003). The sulfate is more resistant to hydrolysis than either isomer (Guerin 2001). The α- and β-isomers are resistant to photolysis in water (Goebel et al. 1982; U.S.EPA 2002) while the sulfate and diol metabolites are susceptible to photolysis (http://www.inchem.org/documents/hsg/hsg017.htm).

Endosulfan sulfate is the major oxidation product expected in water and sediment under aerobic conditions as a result of biological transformation by a variety of fungi and bacteria (Navarro et al. 2000; NRCC 1975; Shivaramaiah et al. 2005; Sutherland et al. 2000, 2004; U.S.EPA 2002). The α-isomer is more prone to oxidation to endosulfan sulfate than the β-isomer in water (Walse et al. 2003).

In general, the rates of hydrolysis and photolysis in water of the β-isomer are more rapid than the α-isomer (Sutherland et al. 2004). When volatilization is controlled and the major transformation pathways are abiotic the half-life of the β-isomer is less than with the α-isomer

(Sutherland et al. 2004). In typical sediment–water systems, where volatilization is a major transformation route, the α-isomer has a shorter half-life than the β-isomer. Increased rates of volatilization by the α-isomer are reflected in the atmosphere where this isomer is usually higher in concentration than the β-isomer (Yao et al. 2008). Therefore, in an aquatic system of the two isomers, the β-isomer is typically more persistent.

The physical–chemical and environmental fate chemistry characteristics provide support for the reasons why the α-isomer and the sulfate are the two most prevalent endosulfan substances detected in animal tissue in locations distant from initial agriculture application sites of technical grade material (GFEA 2007).

Mode of toxic action

Endosulfan is a neurotoxicant since it binds to the gamma-aminobutyric acid (GABA)-gated chloride channel receptor inhibiting GABA-induced chloride flux across membranes in the central nervous system (Ffrench-Constant 1993, 2000; Hassall 1990; IRAC 2007).

Ecosystems at risk

The South Florida freshwater ecosystem was the focus of this aquatic risk assessment (Fig. 1). South Florida covers 16 counties from south of Orlando, in the center of the State to the Florida Keys at the southern tip and is under the jurisdiction of the SFWMD (Fig. 1). It encompasses a variety of freshwater habitats and is dominated by the watersheds of the Kissimmee River and Lake Okeechobee in the north and the Everglades in the south and includes ~2,250 km (1400 mi) of freshwater canals (Rand and Gardinali 2004). The subtropical climate, long crop-growing season, application frequency, and multitude of pesticides used (e.g., mosquito and termite control, golf courses and landscape management) along with the acute toxicity and potential residual activity of endosulfan isomers and the sulfate renders them particularly hazardous in South Florida ecosystems.

Ecological effects

The aquatic toxicity test database on endosulfan is composed of studies with primarily technical grade endosulfan (α- plus β-isomers) in water and limited data on α- and β-isomers and the transformation product, endosulfan sulfate. A review of the acute toxicity data for endosulfan (technical) to aquatic organisms shows a concentration range of 0.1 (striped bass, *Morone saxatilis*) to 166 (*Daphnia magna*) μg/L (U.S.EPA 2002, 2007). Fish generally have the lowest acute toxicity values for endosulfan (technical) compared to invertebrates (i.e., arthropod and non-arthropod), which is also supported in a recent investigation using 88 acute 48 h- and 96 h- LC50 laboratory studies (see Appendix 1 in Hose and Van den Brink 2004). Sparling and Fellers (2009) determined an LC50 of 0.55 μg/L for endosulfan (technical) and larvae of the amphibian (*Rana boylii*).

The U.S.EPA (2007) risk assessment document shows that the 96 h-LC50 of endosulfan sulfate (3.8 μg/L) for bluegill sunfish (*Lepomis macrochirus*) is similarly toxic to technical endosulfan (1.7 μg/L). Furthermore, recent 96 h-LC50 studies show that native South Florida freshwater fish are also sensitive to endosulfan sulfate (96 h-LC50s = 1.9–3.0 μg/L) and the slope of the concentration–response curves for LC50s is steep, indicating that large increases in species mortality are associated with relatively small increases in endosulfan sulfate water concentrations to which the fish are exposed (Carriger et al. in preparation).

Alpha and beta isomers are also acutely toxic (low μg/L range) to aquatic organisms and it has been shown that the α-isomer is more toxic than the β-isomer, and that α- + β-endosulfan and endosulfan sulfate are both toxic to rainbow trout (*Oncorhynchus mykiss*) and the amphipod *Hyalella azteca* (Wan et al. 2005). There is limited toxicity data for aquatic organisms on the effects of chronic exposure to endosulfan (technical). The limited chronic effects data indicate that the NOECs for the fathead minnow (*Pimephales promelas*) and the cladoceran, *Daphnia magna* were 0.2 and 2.0 μg/L, respectively (U.S.EPA 2002). The most sensitive endpoints in these studies were reduced growth and survival. There were no chronic aquatic toxicity data with the isomers and endosulfan sulfate.

Acute exposure to endosulfan, (technical) at environmentally realistic surface water concentrations, was observed to produce effects on relevant biological parameters typically measured in partial or full life cycle chronic fish toxicity tests, where organisms are exposed for extensive periods of time. For example, short-term, pulse exposure (24 h) to endosulfan (0.01–1.0 μg/L) on the medaka fish (*Oryzias latipes*), increased egg hatching time, decreased fry size and decreased mobility (Gormley and Teather 2003). Upon reaching sexual maturity fish displayed increased or decreased egg production, depending on exposure time and increased hatching time of eggs. Effects were not dose-dependent, with eggs or fry exposed to the intermediate concentration (0.01 μg/L) of endosulfan typically producing the greatest response. Willey and Krone (2001) also found that 10 days of exposure of zebrafish (*Danio rerio*) embryos up to 1 μg/L endosulfan resulted in higher incidence of mortalities. Recently, biological changes over two generations were noted in crimson-spotted rainbowfish (*Melanotaenia fluviatilis*), a model Australasian freshwater fish, following a 4-h exposure to

endosulfan concentrations as low as 1 μg/L (Holdway et al. 2008). Significant correlations were found between reproductive and physiological parameters for the first parental (F_0) generation. For example, percentage hatch after exposure, steady spawning and number of infertile eggs for exposed female adults were significantly different from controls. There were also significant reductions in the percentage hatch of F_1 eggs collected from F_0 adults. The NOEC for this study was <1.0 μg/L.

Acute, pulsed exposures are probably the most common scenario in surface waters for endosulfan after application. However, in sediment, where the potential residual activity of the α- and β-isomers and the sulfate increases, chronic exposure scenarios are more likely, especially adjacent to agricultural lands during South Florida's long growing season. Under the latter scenario, chronic toxicity data with exposures from sediment are needed for better risk predictions.

A summary of aquatic field and in situ toxicity studies with endosulfan applied either directly, or through actual application concluded that mortality of fish and invertebrates is a real concern when endosulfan is applied in agricultural areas near aquatic systems (Carriger and Rand 2008a). For further information on the characteristics of the isomers and transformation products, environmental fate (transformation and persistence), aquatic toxicity and measured concentrations in non-target areas see the summaries by Carriger et al. (2006), Carriger and Rand (2008a, b), GFEA (2007), Goebel et al. (1982), NRCC (1975), Sutherland et al. (2004) and U.S.EPA (2002, 2007).

Assessment and measurement endpoints

For this aquatic ecological PRA in surface water, we selected survival, growth and reproduction of invertebrates and fish in South Florida freshwaters as the environmental value we want to protect. The measurement endpoints considered all laboratory toxicity data related to survival, growth and reproduction of organisms.

For tissue concentrations (whole-body) of endosulfan from fish collected (Gardinali et al. in press) in South Florida we selected survival of fish species as the environmental value worthy of protection. The measurement endpoint considered laboratory and field tissue data related to mortality which were compared to the aforementioned measured concentrations of total endosulfan in fish tissue.

Risk analysis

Exposure assessment

The exposure assessment in Tier 2 (PRA) was designed to examine the co-occurrence of three potential stressor exposures (i.e., α- plus β-endosulfan, endosulfan sulfate alone, and α- plus β-endosulfan plus endosulfan sulfate (total)) from surface water of South Florida aquatic ecosystems with the ecological receptors in question, for sites that contained stressors with HQs greater than 1. Probability distributions of exposure data for the stressors were developed based on their measured surface water concentrations at sites in South Florida surface waters (Fig. 1) to assess spatial trends. Exposure data for the stressors were taken from the SFWMD PEST program database from April 1992 to December 2007 (R. Pfeuffer, SFWMD; personal communication). For summation of isomers and total endosulfan, concentration values less than detection limits were converted to a zero.

Measured surface water concentrations were used to develop cumulative log-logistic distributions, using least squares regression (Giddings et al. 2000). The surface water concentrations were ranked from lowest to highest and assigned a centile ranking j at each site using the following equation

$$(j \times 100)/(n + 1) \quad (1)$$

where j is the rank number assigned to the data point and n is the total number of observations (including non-detects) at each site (Hall and Gardinali 2004). The non-detects were used to calculate ranks and assumed to be censored at the lower end of the distribution (Giddings et al. 2000; Hall et al. 1998). When four or more surface water concentration values were detected, the data were plotted and the 90th centile exposure concentrations were estimated (i.e., for total endosulfan (α- plus β-endosulfan isomers plus endosulfan sulfate); α- plus β-endosulfan isomers and endosulfan sulfate alone) from log-logistic distributions fit to the exposure data. The estimated 90th centile concentration was used as the "exposure descriptor" (Solomon et al. 1996). The 90th centile concentration estimate assumes that 90% of the exposure concentration samples will be below that descriptor if it comes from an exposure distribution which is unbiased and which accurately represents the concentrations found for a location during a given time period (Giddings et al. 2005).

An exposure analysis was also conducted based on whole body tissue concentrations of total endosulfan measured in live fish collected from South Florida freshwater marshes and canals. Data for endosulfan in fish tissue came from Gardinali et al. (in press). Fish tissue data for endosulfan (μg/kg, ww) were separated into distributions by species and geographic locations where fish were collected (Fig. 2; Tables 5, 6). To calculate 90th centile concentration estimates for fish tissue it was assumed that data were log-logistically distributed.

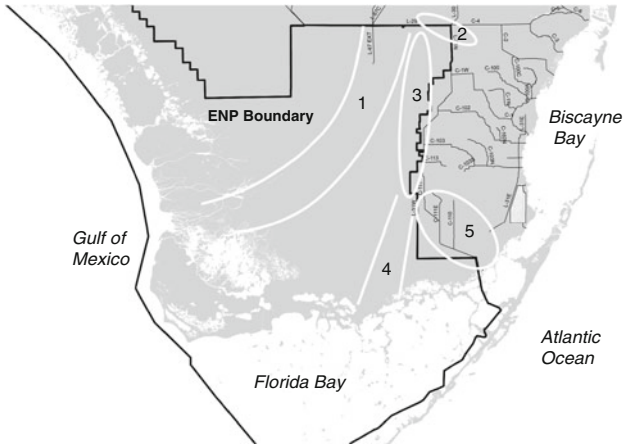

Fig. 2 Location groupings of endosulfan fish tissue data in South Florida. 1 = Shark River Slough, 2 = Tamiami Trail, 3 = Eastern boundary, 4 = Taylor Slough, 5 = C-111. ENP = Everglades National Park

Effects assessment

We used toxicity effects distributions for comparisons to both the surface water and fish tissue concentration distributions. The toxic effects distributions are discussed below.

For comparing toxic effects distributions to surface water exposure distributions, acute (LC50/EC50) and chronic (NOEC) laboratory toxicity data for water effects from technical grade endosulfan and endosulfan sulfate to freshwater species were used to develop SSDs. The SSD is used in ecological risk assessment to determine the concentration of a chemical stressor that is protective of most species (e.g., 90–95%) in the environment. They are typically constructed by fitting a cumulative distribution function to a plot of species data from laboratory toxicity tests against rank assigned centiles. Hose and Van den Brink (2004) indicate that SSD curves and safety data for endosulfan based on single species laboratory toxicity tests will be protective of aquatic organisms in outdoor mesocosm systems. Separate acute SSDs were developed for fish and arthropods in this assessment. However, fish were generally more sensitive than arthropods and different fish species showed similar acute toxicity to endosulfan (technical) (Carriger and Rand 2008a, b). Plant groups were not considered in this analysis due to the limited amount of data. For chronic SSDs, all toxicity tests for endosulfan (technical) were combined into one SSD because of limited chronic toxicity data. No chronic SSDs were created for endosulfan sulfate because there are only two chronic tests with aquatic organisms for this metabolite. At least four species observations were required to construct a SSD but more was considered optimal.

Toxicity data for technical endosulfan in the SSDs were developed primarily from the U.S.EPA AQUIRE database (http://www.epa.gov/ecotox). Chronic toxicity data for technical endosulfan were also taken from the literature and a RIVM document used for establishing WQC in the Netherlands (Van de Plassche et al. 1994). Endosulfan sulfate toxicity data came from the published literature, U.S.EPA RED document addendum (2007) and recent acute toxicity data developed in our laboratory (Carriger et al. in preparation).

Effects data were screened for usage in SSDs. Toxicity endpoints that could clearly be related to changes in population structure such as survival, growth, development and reproduction were used in the development of SSDs. Each species was only used once in a distribution. Where multiple acceptable toxicity values were available for a species, the geometric mean of all values was used in the SSD. Toxicity tests that reported nominal and/or measured concentrations were also included since Wan et al. (2005) observed that acute toxicity values based on measured and nominal concentrations of endosulfan did not significantly differ in their tests. In addition, species were selected whether they were residents of North America or not. Hose and van den Brink (2004) observed that SSDs constructed with endosulfan and Australian species were similar to SSDs created with species elsewhere. Tests using temperate, sub-tropical and tropical fish species were also considered. Cold water and warm water fish had similar toxicity results when the data were compared therefore all test results were included in the distribution. Including more species data (i.e., a higher n value) in a SSD typically creates more statistically robust estimates.

All effects (toxicity) data used for SSDs were assumed to fit a log-logistic distribution and ranked and plotted using the same methods as described for the exposure data. A log-logistic distribution was chosen due to its established usage and ease of calculations for the multiple stressor assessment with endosulfan and endosulfan sulfate described later. However, effects data were right censored where toxicity data above the water solubility of the compound were assigned a rank but not included in the regression. This was found to reduce bias in a previous SSD assessment (Solomon et al. 2001). The toxicity "effects benchmarks" selected in this risk assessment were the 10th and 5th centile concentration estimates (i.e., the HC5 or the HC10 or hazardous concentration expected to exceed 5 or 10% of the toxicity values, respectively) from the SSD (Hall et al. 1999).

Effects data for total endosulfan fish tissue residues were also collected. The literature indicates that concentrations of the toxicant (i.e., in whole body) in aquatic organisms are associated with biological response endpoints (e.g., mortality) (McCarty and MacKay 1993; Escher and Hermens 2002, 2004). This implies that the whole body

concentration of an organic chemical is a reasonable first approximation of the amount of chemical reaching the site(s) of toxic action which is responsible for the adverse response (e.g., mortality) observed. Therefore, lethality is expected in an exposure regime when fish reach or exceed the critical body residue.

The primary sources of information for fish tissue-residue effects data were available for total endosulfan from the U.S. Army Corps of Engineers/U.S. Environmental Protection Agency Environmental Residue Effects Database (http://el.erdc.usace.army.mil/ered/) and Jarvinen and Ankley (1999). Data were screened for whole body tissue residues of total endosulfan that were associated with lethalities in fish. After cross-checking the two databases, it was determined that data were available from two sources: Matthiessen et al. (1982) and Schimmel et al. (1977). The former study measured tissue concentrations in dead fish, in the field, after endosulfan was sprayed in Okavanga Delta, Botswana, in 1978 to control tsetse flies. Fish were collected within 24 h in shallow areas around the region sprayed. Specimens collected were primarily juveniles with the exception of one species of fish (*Pseudocrenilabrus philander*). Whole-body lethal dose information was available for a catfish genus (*Clarias* sp.), mouthbrooders (*Haplochromis* sp.), dwarf copper-mouth brooder (*P. philander*), cichlids (*Serranochromis* sp.), and pooled samples of dead cichlids (*Sarotherodon* sp.) and tilapias (*Tiliapia* sp.). The Schimmel et al. (1977) study measured tissue concentrations of endosulfan in estuarine fish (pinfish, *Lagodon rhomboides*, spot, *Leiostomus xanthurus*, and striped mullet, *Mugil cephalus*) from three laboratory toxicity tests with technical endosulfan. The fish tissue analyzed originated from fish in treatments that survived acute toxicity exposures. All available tissue-residue effects data were taken from both references. In the Matthiessen et al. (1982) paper, direct estimates of endosulfan in fish tissue were used as an effect endpoint (LD or lethal dose). For the Schimmel et al. (1977) studies, the fish tissue endpoints were taken from treatments where the proportion of fish found dead was lower than the LD50 (i.e., LD35 for two fish species, LD40 for one fish species) with effective concentrations of 31, 272, and 360 µg/kg ww for each respective fish species and lethal dose associated with a proportion of mortality.

All of the above lethal effects from whole body tissue data of total endosulfan were used for a lethal (whole body) tissue SSD and were assumed to fit a log-logistic distribution and ranked and plotted using the same method as described for the aquatic toxicity data. We then calculated "tissue effects benchmarks" for total endosulfan which were the 10th and 5th centile concentration estimates from the SSD.

Risk characterization

Individual and multiple stressors

Risk was assessed by comparing the overlap of the insecticide distributions of the surface water exposure concentrations for three endosulfan stressors [i.e., α- plus β-isomers plus endosulfan sulfate (total); α- plus β-isomers, and endosulfan sulfate (alone)] for sites and the SSDs for arthropods and fish. For acute risks to arthropods, fish and all species combined (i.e., arthropods and fish), the estimated 90th centile surface water concentrations for exposure were compared to the estimated 10th and 5th centile concentrations of the acute SSD (SETAC 1994). For chronic risks to aquatic organisms, the estimated 50th centile surface water concentrations for exposure were compared to the estimated 10th and 5th centile concentrations of the chronic SSD for all species combined (Traas et al. 2002). The 50th centile concentration was chosen as a comparison to chronic SSDs because it might be more representative of background concentrations as 50% of the exposures are anticipated to be above or below this level at a site. The 90th centile concentration is located at the upper portion of the distribution and would be expected to be encountered less frequently (10% of the time at a site) and would represent episodic or pulsed exposures. The estimated 90th or 50th centile exposure concentrations applied to the acute and chronic SSDs were used to calculate the potentially affected fraction (PAF) of species toxicity values (Klepper and van de Meent 1997). The PAF approach was used to assess risk both individually and in joint action (multiple substance PAF = msPAF) (Traas et al. 2002).

For the individual approach, we evaluated PAFs for the three potential stressor exposure scenarios (total endosulfan, α + β-endosulfan, endosulfan sulfate). Total endosulfan acute and chronic PAFs were estimated by applying the 90th centile or 50th centile concentration estimate from total endosulfan exposure distributions (summation of α-endosulfan + β-endosulfan + endosulfan sulfate) to the SSDs for technical endosulfan. The α- + β-endosulfan acute PAF was estimated by applying the 90th centile concentration estimate from the sum of α- + β-endosulfan isomers exposure distribution to the acute SSD for technical endosulfan. The endosulfan sulfate acute PAF was estimated by applying the 90th centile concentration estimate from the endosulfan sulfate exposure distribution to the endosulfan sulfate acute SSD. From the intersection of the 90th centile or 50th centile exposure concentration estimate on the acute or chronic SSDs, a fraction of toxicity values expected to be affected, or a PAF value, was calculated.

After we calculated the PAFs from exposure scenarios with α- + β-endosulfan and endosulfan sulfate, we applied a multiple stressor model for PRA (the msPAF model) (Traas

et al. 2002). This model was previously used to estimate potential risks from total endosulfan with other pesticides in the South Florida system by Carriger and Rand (2008b).

The msPAF model is a tool that moves PRA away from focusing exclusively on the potential risk of single chemical exposures. Distributions of toxicity and exposure values for single chemicals are used in the msPAF model and they are then brought together for overall estimates of potential risk. Using the msPAF model, we compared exposure data for endosulfan sulfate and α- + β- endosulfan to their respective SSDs and then brought the individual risk predictions together for an overall estimate of risk, i.e., the msPAF. In the previous sections, the detected values of α-endosulfan, β-endosulfan and endosulfan sulfate were summed for each sample and log-logistic distributions were fit to the sums for a total endosulfan assessment. The msPAF approach bypasses the need to sum endosulfan sulfate with the isomers to create an exposure distribution for total endosulfan. As long as there are sufficient toxicity data, the exposure distributions for both α- + β-endosulfan and endosulfan sulfate are sufficient for characterizing the risk of total endosulfan in the msPAF approach. However, the msPAF does not directly address the likelihood of co-occurrence but inferential-based techniques might be used to extend the msPAF approach for a more comprehensive estimate of ecological risk.

The msPAF approach was used with a concentration addition (CA) model (Traas et al. 2002). Concentration addition was applied under the assumption that the chemicals do have the same mode of action and the effects are additive for all chemicals in a mixture. In CA chemicals do not possess toxicological interaction (e.g., synergism).

To implement the CA version of the msPAF model, the concentration of concern for each chemical at a site was transformed to hazard units (HU) representing the relative potency of the actual measured environmental concentration to an SSD (Traas et al. 2002):

$$HU = \frac{C_{ENV}}{10^{\alpha^*}} \tag{2}$$

where C_{ENV} is an exposure concentration of concern (e.g., the estimated 90th centile surface water concentration from a distribution of exposure) and α^* is the mean of log toxicity data for that particular chemical and can be calculated from the slope, β, and intercept, α, of a log-logistic distribution with $\alpha^* = -\alpha/\beta$. There are no units for HU and the transformation of exposure concentrations to HUs is similar to the scaling of toxic units in classical applications of CA theory (Traas et al. 2002).

The estimated parameter β^* can be calculated from the slope, β, of a log-logistic distribution as $\beta^* = 1/\beta$. For both technical endosulfan and endosulfan sulfate SSDs the β^* parameters are joined together (averaged) and substituted into the following equation (from Traas et al. 2002):

$$PAF_{TMoA} = \frac{1}{1 + e^{-\log(\sum HU_{TMoA})/\beta^*_{TMoA}}} \tag{3}$$

The sum of the HUs from a particular centile concentrations of the relevant exposure distributions can be substituted into the equation above. For the msPAF values for CA, HUs were summed for distributions of (α- + β-endosulfan) and endosulfan sulfate at S-177 and S-178. Fish SSDs for technical endosulfan and endosulfan sulfate were used to run the model under the assumption that, as a grouping, fish share a similar molecular site of action to endosulfan. For the calculation of HUs for endosulfan sulfate and the α + β isomers, the parameters α^* from the fish SSD for endosulfan sulfate and for technical endosulfan were applied. However, only the parameter β^* from technical endosulfan was used since the steeper slope observed from endosulfan sulfate might bias the results due to the low number of available freshwater fish toxicity tests used to construct the distribution ($n = 5$). As a comparison to the risk output from the total endosulfan assessment, the joint risk from individual distributions of α- + β-endosulfan and endosulfan sulfate at S-177 and S-178 was evaluated.

In order for the Traas et al. (2002) modifications to CA to work, the slopes of the SSDs for the separate chemicals (i.e., β^*) must be similar. De Zwart et al. (2002) found that SSDs created for separate compounds with a shared mode of action have similar slopes. Therefore, the slopes of separate SSDs with the same mode of action are averaged together under the assumption that they are similar in the msPAF-CA model.

Joint probability curves

The last step in the PRA used joint probability curves (JPCs; or exceedence profiles) for endosulfan at sites which displayed the highest PAF values. Joint probability curves were constructed by applying the various centiles of the exposure distribution for endosulfan (α- + β-) and endosulfan sulfate to log-logistically derived effects distributions (SSDs) for arthropods and fish following the PAF methodology of Traas et al. (2002). The JPCs characterize "the relationship between magnitude of effect and the probability of occurrence for that effect" (ECOFRAM 1999). Joint probability curves were used to determine the proportion of toxicity values in the SSD that were exceeded over the duration of exposures at sites S-177 and S-178 from 1992 to 2007. This approach provides a means of comparing relative potential risk at sites when the toxicity data base and exposure information are adequate. For each potential risk scenario, risks were also described using area under curves of the resulting JPCs. Area under the curve calculations were made in order to completely compare the exceedence of the

toxicity data by the exposure data for each risk scenario. When the JPC likelihood and exceedence data are input as proportions rather than percentages, the area under the curve can be used as a surrogate for the mean risk of a scenario (Giddings et al. 2005; Aldenberg et al. 2002). Areas under curves were calculated using the SigmaPlot 11.0 Area Below Curves macro (Systat 2008) following methods described in Giddings et al. (2005). The trapezoidal rule was utilized to calculate the areas (Systat 2008):

$$Area = \sum_{i=0}^{n-1} [y_i(x_{i+1} - x_i) + 0.5(y_{i+1} - y_i)(x_{i+1} - x_i)] \quad (4)$$

The resulting area estimates were multiplied by 100 to describe the mean risk as a percentage rather than as a proportion (Giddings et al. 2005).

Fish tissue

The log-logistic distribution of lethal body (whole) burden endosulfan (total) data taken from the literature were compared to log-logistic distributed tissue residue concentrations of endosulfan (total) in South Florida fish (Gardinali et al. in press) grouped either by species or geographic location. Risk was characterized for a species or location if a sufficient number of fish species or fish within a location were found with measured endosulfan concentrations ($n \geq 4$). Fish tissue residue data were available in Shark River Slough (1), Tamiami Trail (2), the eastern boundary of the Everglades National Park (ENP) (3), Taylor Slough (4), and the C-111 canal system (5) (Fig. 2). Risks at geographic locations were estimated for Shark River Slough (1), the eastern boundary of the ENP (3), Taylor Slough (4), and the C-111 canal system (5). The n value was either too low or the distribution was too uncertain to estimate risks at Tamiami Trail (2). The distributions of fish tissue endosulfan residues by species and location were used to estimate a 90th centile concentration and predict the PAF of that concentration for lethal body burdens from the literature. Comparisons were done on a wet weight basis for both exposure and effects. Joint probability curves were also created to represent exceedences of the distribution of lethal dose residues by measured concentrations of endosulfan (total) in fish tissues for various species and geographic locations.

Results and discussion

Tier 1

In the first tier, data were screened for detections of endosulfan (α-, β- and endosulfan sulfate) in surface waters of freshwater canals from the SFWMD pesticide monitoring program. Measured concentrations of endosulfan from the SFWMD monitoring program were compared to the U.S.EPA's WQC for α- and β-endosulfan (i.e., the CCC and the CMC). The state of Florida WQC guidelines (Class III freshwater uses) are equivalent to the U.S. EPA's CCC for endosulfan. Endosulfan sulfate was also compared to the CCC and CMC and then summed with α- + β-endosulfan for comparisons of total endosulfan.

The majority of endosulfan detections and α and β water quality violations were found at locations in the C-111 system (i.e., S-177 and S-178; Fig. 1), which is a buffer zone near the southeastern boundary of ENP in South Florida which separates the C-111 from highly productive subtropical agricultural lands and urban development to the east. At S-177, α-endosulfan was detected 21 times, β-endosulfan was detected eight times and endosulfan sulfate was detected seven times. The CCC was not exceeded for the summation of the $\alpha + \beta$ isomers or for endosulfan sulfate. For total endosulfan, the CCC was exceeded twice at S-177. The CMC was not exceeded. At S-178, α-endosulfan was detected 31 times, and β-endosulfan was detected 23 times. However, unlike S-177, endosulfan sulfate was found 50 times, approximately two times that of the isomers. Chronic WQC (CCC) exceedences for α- + β-endosulfan were only found nine times. However, CCC exceedences for total endosulfan ($\alpha + \beta$ + endosulfan sulfate) were found 34 times. Acute WQC (CMC) exceedences for α- + β-endosulfan were only observed once while CMC exceedences for endosulfan sulfate and total endosulfan were found seven and eight times, respectively. Endosulfan sulfate was detected less than 20% of the time at all sites with the exception of S-178 where it was found in more than 65% of the samples.

Downstream from S-178, S-18C had comparatively few detections of endosulfan. The CCC was exceeded once for endosulfan sulfate and once for total endosulfan at S-18C from the same sample. Outside of certain sites monitored regularly between 1992 and 2007, data were inconsistent. Some sites such as S-332, S-332B, S-332C, and S-332D had samples taken from different water quality projects by the SFWMD. Sites where endosulfan was found sporadically (<4 overall detections) included BERMB3 (only four samples taken), C51SR7 (sampled only between 1997 and 1999), FECSR78, G-211, L3BRS, GORDYRD, L40-1, S-355A, S-6, S-7, S-79, and S-8. Additional sites had non-detections for endosulfan in several samples and were not regularly monitored. Summing the isomers with endosulfan sulfate (total endosulfan) for comparisons to the WQC sometimes showed more exceedences.

From the number of detections ($n \geq 4$), nine sampling sites (Acme1DS, G94D, S-176/S-332D, S-177, S-178, S-18C, S-331, S-332, and S-80) were chosen for higher tier

analysis. Four or more detections were the minimum deemed necessary to create a log-logistic regression of exposure data. Acme1DS is located adjacent to the Everglades Agriculture Area and on the boundary of Water Conservation Area 1. S-176, S-177, S-178, S-18C, and S-332 are located in or near the C-111 canal system in the southern portion of the state. S-331 is north of the C-111 basin on the L-31N canal. S-80 is in St. Lucie County and discharges water into the South Fork of the St. Lucie River from C-44 which is connected to Lake Okeechobee. Of the canals sampled, S-18C currently inputs water to the ENP and S-332D controls water levels in L-31W and the S-332D detention basin which discharges into the ENP.

Tier 2

Risk analysis (exposure)

Table 1 presents the log-logistic exposure regression statistics of total endosulfan ($\alpha + \beta$ endosulfan and endosulfan sulfate), $\alpha + \beta$ endosulfan and endosulfan sulfate (alone) for each of nine sites. The highest 90th centile concentration estimates were all found at S-178 (0.31 µg/L for total; 0.05 µg/L for $\alpha + \beta$ endosulfan; 0.30 µg/L for endosulfan sulfate) in C-111. The STORET database for surface water monitoring data in the U.S. indicates a similar (0.31 µg/L) 90th centile value (U.S.EPA 2002). S-178 is a site, surrounded by agriculture, near a gated structure located on a shallow drainage canal which controls flows from Loveland Slough to the mainstem of C-111. The next highest 90th centile concentration estimates for total endosulfan and the sum of the $\alpha + \beta$ isomers were found at S-177. The second highest 90th centile concentration estimate for endosulfan sulfate was found at Acme1DS but detections of endosulfan sulfate were infrequent at Acme1DS (5 detected concentrations out of 43 samples). Distributions with five or fewer detected concentrations tended to be less robust.

Table 2 presents the statistics for the log-logistic distribution of total endosulfan concentrations in fish tissue by species. The highest and next highest 90th centile concentrations for endosulfan tissue estimates were in flagfish (*Jordanella floridae*) and marsh killifish (*Fundulus confluentus*). Mosquitofish (*Gambusia affinis*) were captured more than any other fish species and their 90th centile concentration estimate was the third highest. The 90th centile concentration for endosulfan in the tissue of the Mayan cichlid (*Cichlasoma urophthalmus*), a non-native fish species, was similar to that in mosquitofish. For sailfin molly, six samples (i.e., five detections) contained total endosulfan whole body residues ranging from 3.4 to 99.8 µg/kg (ww). The latter value was the highest detected concentration of endosulfan found in any of the fish samples. However, the log-logistic distribution did not fit the data well and we did not include it in our exposure analyses.

Table 3 presents the statistics for the distribution of total endosulfan concentrations in fish tissue by region. The 90th centile concentration estimate for total endosulfan in fish

Table 1 Total endosulfan ($\alpha + \beta$+sulfate), α- + β-endosulfan, and endosulfan sulfate exposure statistics for log-logistically distributed concentrations

Exposure grouping	Site	Number of times analyzed	Number of detections	Slope	Intercept	r^2	*p*-value for the slope	90th centile (µg/L)
Total endosulfan	Acme1DS	43	5	1.465	5.535	0.81	3.66E−02	0.0053
	G94D	44	4	0.957	4.325	0.995	2.33E−03	0.0060
	S-176	68	9	2.459	7.637	0.98	5.41E−07	0.0061
	S-177	68	23	2.625	6.370	0.88	5.54E−11	0.0257
	S-178	68	50	2.379	3.395	0.92	1.82E−28	0.3136
	S-18C	68	7	1.893	6.687	0.92	5.98E−04	0.0042
	S-331	68	4	2.389	8.417	0.98	1.01E−02	0.0025
	S-332	43	5	2.155	7.417	0.81	3.75E−02	0.0038
	S-80	68	4	0.971	5.233	0.84	8.57E−02	0.0007
α- + β- endosulfan	S-176	68	9	2.699	8.170	0.99	5.94E−08	0.0061
	S-177	68	22	2.801	6.904	0.84	1.56E−09	0.0209
	S-178	68	29	2.222	5.035	0.93	5.48E−17	0.0528
	S-18C	68	5	5.702	15.869	0.94	6.37E−03	0.0040
	S-332	43	5	2.155	7.417	0.81	3.75E−02	0.0038
Endosulfan sulfate	Acme1DS	43	5	1.791	6.373	0.66	9.27E−02	0.0047
	S-177	68	7	1.611	6.110	0.92	7.08E−04	0.0037
	S-178	68	44	2.192	3.353	0.80	2.47E−16	0.2969

Table 2 Endosulfan (total) fish tissue (whole body) residue 90th centile concentration estimates from log-logistically distributed data by species

Species	Number of times analyzed	Number of detections	Slope	Intercept	r^2	*p*-value for the slope	90th centile (μg/kg ww)
All species	85	74	2.208	−1.077	0.97	5.46E−56	30.4
Flagfish (*Jordanella floridae*)	10	7	1.823	−1.135	0.96	1.29E−04	67.2
Jewel cichlid (*Hemichromis bimaculatus*)	5	5	3.367	0.116	0.97	2.63E−03	4.2
Mayan cichlid (*Cichlasoma urophthalmus*)	11	9	1.742	−0.469	0.94	1.33E−05	33.9
Mosquitofish (*Gambusia affinis*)	15	14	2.331	−1.500	0.97	1.20E−10	38.6
Marsh killifish (*Fundulus confluentus*)	10	9	2.866	−2.632	0.96	2.94E−06	48.4
Sunfish	13	11	2.410	0.150	0.93	1.36E−06	7.1

ww wet weight

Table 3 Endosulfan (total) fish tissue (whole body) residue 90th centile concentration estimates from log-logistically distributed data by location

Location	Number of times analyzed	Number of detections	Slope	Intercept	r^2	*p*-value for the slope	90th centile (μg/kg ww)
All freshwater sites	85	74	2.208	−1.077	0.97	5.46E−56	30.4
C-111	17	16	3.905	−0.687	0.95	2.85E−10	5.5
Eastern boundary	42	41	2.706	−2.563	0.95	2.43E−27	57.5
Shark River Slough	11	5	3.131	0.588	0.97	1.83E−03	3.3
Taylor Slough	9	9	1.943	−0.912	0.90	8.77E−05	39.8

ww wet weight

tissue at the eastern boundary of the ENP was the highest and ten times greater than the 90th centile concentration estimate at the C-111 system. The 90th centile fish tissue concentration estimate for total endosulfan in Shark River Slough was nearly 18 times lower than at the eastern boundary of the ENP. Tamiami Trail had three measured endosulfan concentrations in fish tissue out of six samples which ranged from 0.59 to 5.3 μg/kg ww. Located in the southern end of the eastern boundary of the ENP, Taylor Slough had the second highest 90th centile concentration estimate for total endosulfan in fish tissue at 39.8 μg/kg ww. This was largely due to a site where several fish species were collected located at the southern end of the eastern boundary of the ENP in Taylor Slough. Other sites from Taylor Slough had individual measured concentrations that were all below the 90th centile concentration estimates for other geographic locations.

In fish tissue, endosulfan sulfate was the major contributor to all total endosulfan concentrations and it was detected at greater frequencies than α- and β-endosulfan (Gardinali et al. in preparation). Examples of long-term retention of the sulfate metabolite are present in the literature. A recent dietary bioaccumulation study [92-d phase of feeding endosulfan-enriched diet (uptake phase) followed by a 56-d phase of control diet (depuration phase)] with Atlantic salmon (*Salmo salar*) showed: higher uptake and lower elimination for β-isomer than α-isomer resulting in higher dietary bioaccumulation of β-endosulfan (than α-endosulfan) from feed to fillet (Berntssen et al. 2008). Despite the fact that α- and β-isomers were rapidly eliminated during the depuration period; and although endosulfan sulfate, the metabolic breakdown product (not detected in feed or control fish), contributed only 1.2% of the total endosulfan, it remained unchanged in the organism during the 56-d depuration phase. In water and sediment, endosulfan sulfate is the major oxidation product typically found under aerobic conditions (Shivaramaiah et al. 2005; U.S.EPA 2002). Therefore, based on the likelihood of exposures of aquatic organisms from water and sediment and its propensity to remain in tissue, it is not surprising that endosulfan sulfate was the major endosulfan product detected in the tissue analyzed by Gardinali et al. (in preparation).

Risk analysis (effects)

A summary of the statistics for log-logistically distributed freshwater acute and chronic toxicity SSDs for endosulfan (technical) and acute toxicity SSDs for endosulfan sulfate, including 10th and 5th centile concentration estimates are presented in Table 4. When the term "all species" is used it indicates that the results of all toxicity tests were grouped together into one SSD to obtain 10th and 5th centile concentration estimates for effects. The chronic SSD for

Table 4 Log-logistic species sensitivity distribution statistics for endosulfan aquatic toxicity data by test duration and species

Test exposure condition	Number of different species	β (Slope)	α (Intercept)	r^2	p-value for the slope	10th centile (µg/L)	5th cenile (µg/L)
Technical endosulfan–acute all	64[a]	1.250	−1.476	0.90	1.44E−32	0.26	0.067
Technical endosulfan–acute fish	36	2.435	−0.724	0.98	6.64E−30	0.25	0.12
Technical endosulfan–acute arthropods	25[b]	1.316	−1.790	0.97	1.56E−18	0.49	0.13
Technical endosulfan–chronic	6[b]	0.934	−0.957	0.85	8.93E−03	0.047	0.0074
Endosulfan sulfate–acute all	7[c]	4.334	−2.164	0.95	1.73E−04	0.98	0.66
Endosulfan sulfate–acute fish	5	7.595	−2.785	0.99	1.71E−04	1.2	0.95

[a] Fifteen additional species LC/EC50s were above the water solubility of endosulfan

[b] One additional species LC/EC50 was above the water solubility of endosulfan

[c] Two additional species LC/EC50s were above the water solubility of endosulfan

technical endosulfan had the lowest 10th and 5th centile concentration estimates for endosulfan.

For the lethal residue concentration data, three estuarine fish were used from the study by Schimmel et al. (1977) and seven field-collected freshwater fish were used from the study by Matthiessen et al. (1982). The effect values in Matthiessen et al. (1982) were expressed as lethal concentrations in pooled dead fish tissues while the effect values in Schimmel et al. (1977) were from test treatments where proportions of fish died from exposures to technical endosulfan. Both studies measured total endosulfan (i.e., α, β, and sulfate). From Schimmel et al. (1977), three fish were tested and the effect value chosen was from a treatment with a percent death at the LD35 and LD40. Because the catfish (*Clarias* sp.) were grouped into one sample for analysis by Matthiessen et al. (1982), the whole body concentration producing an effect is one number. Likewise, dead *Sarotherodon* sp. and *Tilapia* sp. were pooled for analysis in the same study so a single concentration was used to represent the latter two species in risk characterization. When all eight fish tissue residue lethal response data points were placed in a log-logistic distribution, the 10th centile concentration was estimated to be 30.9 µg/kg ww, the 5th centile concentration was estimated to be 13.2 µg/kg ww, the slope was 2.019, the intercept was -5.207, the r^2 was 0.90, and the p-value for the slope was 0.00074.

Risk characterization

Potentially affected fraction (PAF) Table 5 contains PAF (%) values for the estimated 90th centile exposure concentrations from log-logistic exposure distributions when applied to the acute SSDs. Potential exceedence of 10% of the toxicity values was found at S-178 on the C-111 system where the estimated 90th centile concentration for total endosulfan potentially exceeded 12.5% of the acute freshwater fish toxicity values. For arthropods, at the same site, the estimated 90th centile concentration for total endosulfan potentially exceeded 7.9% of the acute freshwater arthropod toxicity values. In contrast, the estimated 90th centile total endosulfan exposure value at S-177 potentially exceeded 2.0% of the acute arthropod toxicity values and 1.0% of the fish toxicity values. All other sites had less than a 5% exceedence of freshwater acute toxicity values by the estimated 90th centile exposure concentration. Although low (<5%), potential risks for $\alpha + \beta$ endosulfan were higher than for endosulfan sulfate at S-178 and S-177, where sufficient data were available to create exposure distributions. Note that risk from endosulfan sulfate is low because of the steep acute toxicity (mortality) distribution for the sulfate and also because of the minimal exceedences of effects distribution by concentrations in water.

Carriger and Rand (2008b) and Carriger et al. (2006) also observed that the majority of the exceedences of toxicity values for measured concentrations of endosulfan in surface water and sediment were found at S-178. In the U.S.EPA aquatic PRA for application of endosulfan to tomatoes in Florida, they also recognized the high likelihood for mortality in aquatic species following application (U.S.EPA 2002).

Table 6 contains the PAF (%) values for the chronic toxicity exceedences when the estimated 50th centile exposure concentrations were compared to the chronic toxicity distribution of technical endosulfan (for all species toxicity tests combined). Potential chronic risk (>5% PAF value) was only found at S-178 (9.2% PAF value). All other chronic PAF values were below 5%, with S-177 being the only site that exceeded 3%.

Multiple substance potentially affected fraction (msPAF) The results from the acute msPAF risk characterization for freshwater fish and acute exposures to both isomers together (summed for a sample) and endosulfan sulfate alone are shown in Table 7. For the individual PAF values, the

Table 5 Potentially affected fraction (PAF) of the alpha and beta, sulfate, and total endosulfan acute toxicity values at the 90th centile of the exposure distribution and area under curves for joint probability diagrams

Site	Chemical	PAF (%)	90th centile (μg/L)	Area under the joint probability curve (mean risk %)
Acme1DS	Total endosulfan–all species	1.3	0.0053	0.62
	Total endosulfan–arthropods	0.8	0.0053	0.40
	Total endosulfan–fish	0.2	0.0053	0.25
	Endosulfan sulfate–all species	0.0	0.0047	0.00
	Endosulfan sulfate–fish	0.0	0.0047	0.00
G94d	Total endosulfan–all species	1.4	0.0060	0.93
	Total endosulfan–arthropods	0.9	0.0060	0.67
	Total endosulfan–fish	0.2	0.0060	1.08
S-176	Total endosulfan–all species	1.4	0.0061	0.70
	Total endosulfan–arthropods	0.9	0.0061	0.44
	Total endosulfan–fish	0.2	0.0061	0.12
	$\alpha + \beta$ endosulfan–all species	1.4	0.0061	0.72
	$\alpha + \beta$ endosulfan–arthropods	0.9	0.0061	0.45
	$\alpha + \beta$ endosulfan–fish	0.2	0.0061	0.11
S-177	Total endosulfan–all species	3.0	0.0257	1.52
	Total endosulfan–arthropods	2.0	0.0257	1.00
	Total endosulfan–fish	1.0	0.0257	0.49
	$\alpha + \beta$ endosulfan–all species	2.7	0.0209	1.40
	$\alpha + \beta$ endosulfan–arthropods	1.8	0.0209	0.91
	$\alpha + \beta$ endosulfan–fish	0.8	0.0209	0.39
	Endosulfan sulfate–all species	0.0	0.0037	0.00
	Endosulfan sulfate–fish	0.0	0.0037	0.00
S-178	Total endosulfan–all species	10.9	0.3136	5.26
	Total endosulfan–arthropods	7.9	0.3136	3.77
	Total endosulfan–fish	12.5	0.3136	5.07
	$\alpha + \beta$ endosulfan–all species	4.4	0.0528	2.10
	$\alpha + \beta$ endosulfan–arthropods	3.0	0.0528	1.42
	$\alpha + \beta$ endosulfan–fish	2.1	0.0528	1.12
	Endosulfan sulfate–all species	1.2	0.2969	1.62
	Endosulfan sulfate–fish	0.1	0.2969	1.79
S-18C	Total endosulfan–all species	1.2	0.0042	0.54
	Total endosulfan–arthropods	0.7	0.0042	0.34
	Total endosulfan–fish	0.2	0.0042	0.11
	$\alpha + \beta$ endosulfan–all species	1.1	0.0040	0.75
	$\alpha + \beta$ endosulfan–arthropods	0.7	0.0040	0.46
	$\alpha + \beta$ endosulfan–fish	0.1	0.0040	0.07
S-331	Total endosulfan–all species	0.9	0.0025	0.43
	Total endosulfan–arthropods	0.5	0.0025	0.26
	Total endosulfan–fish	0.1	0.0025	0.05
S-332	Total endosulfan–all species	1.1	0.0038	0.52
	Total endosulfan–arthropods	0.7	0.0038	0.32
	Total endosulfan–fish	0.1	0.0038	0.08
	$\alpha + \beta$ endosulfan–all species	1.1	0.0038	0.52
	$\alpha + \beta$ endosulfan–arthropods	0.7	0.0038	0.32
	$\alpha + \beta$ endosulfan–fish	0.1	0.0038	0.08
S-80	Total endosulfan–all species	0.5	0.0007	0.32
	Total endosulfan–arthropods	0.3	0.0007	0.22
	Total endosulfan–fish	0.0	0.0007	0.18

All species refer to all freshwater species in acute tests. Fish and arthropods refers to only freshwater fish and arthropod species

Table 6 Potentially affected fraction (PAF) of the alpha and beta, and total endosulfan chronic toxicity values at the 50th centile of the exposure distribution and area under curves for corresponding joint probability curves

Site	Chemical	PAF (%)	50th centile (μg/L)	Area under the joint probability curve (mean risk %)
Acme1DS	Total endosulfan	1.1	0.0002	2.01
G94d	Total endosulfan	0.6	0.0000	2.12
S-176	Total endosulfan	2.1	0.0008	2.54
	$\alpha + \beta$ endosulfan	2.2	0.0009	2.64
S-177	Total endosulfan	3.8	0.0037	4.53
	$\alpha + \beta$ endosulfan	3.7	0.0034	4.30
S-178	Total endosulfan	9.2	0.0374	10.8
	$\alpha + \beta$ endosulfan	4.4	0.0054	5.54
S-18C	Total endosulfan	1.4	0.0003	2.00
	$\alpha + \beta$ endosulfan	2.8	0.0016	2.87
S-331	Total endosulfan	1.4	0.0003	1.77
S-332	Total endosulfan	1.5	0.0004	2.00
	$\alpha + \beta$ endosulfan	1.5	0.0004	2.00
S-80	Total endosulfan	0.2	0.0000	0.99

Table 7 Potentially affected fractions (PAF) and concentration addition calculated multiple substance potentially affected fraction (msPAF) for freshwater fish and acute exposures to the α- + β- endosulfan isomers, and endosulfan sulfate at S-177 and S-178

Site	Grouping	Fish PAF (%)
S-177	$\alpha + \beta$ endosulfan PAF	0.8
	Endosulfan sulfate PAF	0.0
	msPAF (CA) with technical β*	0.9
	Total endosulfan PAF	1.0
S-178	$\alpha + \beta$ endosulfan PAF	2.1
	Endosulfan sulfate PAF	0.1
	msPAF (CA) with technical β*	12.2
	Total endosulfan PAF	12.5

Note: β refers to the β isomer for endosulfan and α refers to the α isomer for endosulfan. β* refers to parameters for the log-logistic model used to calculate PAFs (see text for explanation). Total endosulfan was the calculated PAF value after summing the α, β, and sulfate isomers for concentration points in a total endosulfan distribution

90th centile concentration estimate was taken from the exposure distributions for the summation of $\alpha + \beta$ endosulfan and for the distributions for endosulfan sulfate alone at S-177 and S-178 in Table 1. Using the estimated 90th centile concentrations from α- + β-endosulfan and endosulfan sulfate alone in the msPAF approach gave similar risk estimates (0.9% for S-177; 12.2% for S-178) to the PAF estimated from total endosulfan (1.0% for S-177; 12.5% for S-178). Thus, the msPAF approach for α- + β-endosulfan and endosulfan sulfate gave similar results to the total endosulfan PAF approach when a similar slope is assumed between the SSDs for technical endosulfan and endosulfan sulfate and fish.

Joint probability curves (JPCs) The JPCs for S-177 and S-178 and fish and arthropod toxicity values are presented in Fig. 3. S-177 and S-178 were selected because these sites had the highest overall potential risks with the PAF approach. With a JPC, a greater potential for risk can be observed when the curve is further away from the axes (Solomon et al. 2000). At S-177 the greatest potential for acute risk is for freshwater arthropods and total endosulfan exposure. At S-178, the greatest potential for acute risk is for fish and total endosulfan exposure followed by arthropods and total endosulfan. Risk for endosulfan sulfate also becomes more apparent towards the lower end of the exceedence values at S-178. Overall, the JPC for S-178 indicates a much higher exceedence of the exposure distribution with the acute effects distributions for all exposure scenarios than the JPC for S-177.

In Tables 5 and 6, the area under the curve calculations for the JPCs tended to follow the results from the single point PAF approach. However, a few of the risk scenarios had higher or lower area under the joint probability curve values indicating that overall risks might be different than an exposure scenario based on the estimated 90th centile concentration. In particular, several of the risk scenarios at S-178 had lower PAF values and higher mean risk values (areas under the curve) than several others. This is because the percent of toxicity values exceeded by the 90th centile concentration does not describe the risk indicated by the complete distributions. S-178 was also the only site where the mean potential acute risk to fish equaled or exceeded 5% or mean potential chronic risk to all species exceeded 10%.

Tissue Table 8 presents the PAF when the estimated 90th centile measured tissue concentrations of endosulfan (total)

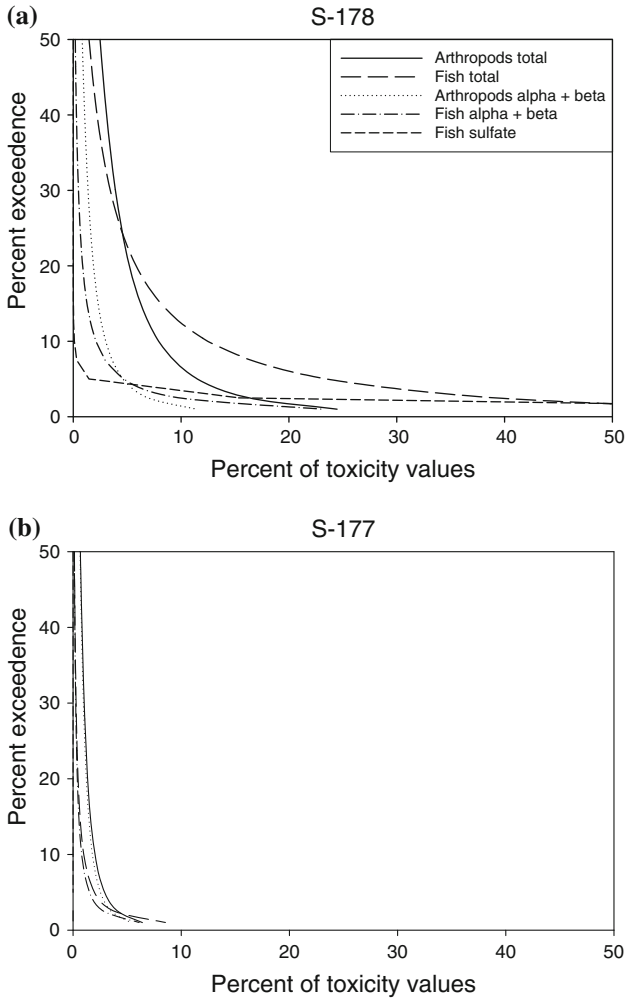

Fig. 3 Joint probability curves for the exposure summations of endosulfan (total endosulfan ($\alpha + \beta$+sulfate), α- + β- endosulfan, and endosulfan sulfate) found in sites S-178 (**a**) and S-177 (**b**) and arthropods and fish

in species collected in South Florida was applied to the SSD for lethal fish tissue residues. The greatest potential risk was found for flagfish, marsh killifish and mosquitofish. Note that the estimated 90th centile concentration of endosulfan in tissue from Mayan cichlid, a non-native species, had a PAF value of 10.8% of lethal toxicity concentrations in fish tissue. Table 9 presents the potential risks for tissue exposure distributions by location instead of by species. The estimated PAF value for lethal whole body residues of fish was greatest in samples collected at the eastern boundary of ENP (16.1%). This value was an order of magnitude greater than C-111(2.4%) or Shark River Slough (1.5%). The potential risk from measured concentration in fish tissue was greater at the eastern boundary of the ENP despite the higher potential risks observed for measured concentrations in water in the C-111 canal system to the south. Taylor Slough also had a relatively high PAF value and mean risk (area under the JPC) calculation. In Tables 8 and 9, the potential risk indicated by the area under the joint probability curve calculations followed the same relative risk patterns for the PAF approach.

Joint probability curves in Fig. 4 confirm that the greatest risk (further distance from the graph axes) is found for flagfish, marsh killifish, mosquitofish, and Mayan cichlid species. A greater risk by geographic location is also observed for the eastern boundary of the ENP in Fig. 5. It is apparent from Fig. 5 that the higher concentrations of endosulfan in tissue of fish in the eastern boundary and Taylor Slough regions are driving the displayed risk for the JPC for all species. The fish tissue risk levels were lower at the C-111 region than the eastern boundary of the ENP. This was not the case for surface water risk levels where potential risk was dominated by a site in the C-111 region (S-178). Potential acute and chronic risks for endosulfan in surface water at S-332 near the eastern boundary of the ENP were low (<2.0% exceedence of toxicity values by the estimated 90th centile concentration). However, nearby sites S-332B, S-332C, and S-332D had a limited monitoring database with samples only taken a few times a year from 2003 to 2006.

Uncertainties

Exposure analysis From 1992 to 2007, endosulfan was applied on a variety of crops in South Florida (e.g., cucumbers, eggplant, pecans, potatoes, snapbeans, tomatoes, and watermelon, to name a few). Considering the size

Table 8 Potentially affected fraction (PAF) of log-logistically distributed lethal body burdens of fish species from the estimated 90th centile of total endosulfan fish tissue concentrations values measured in various fish species throughout South Florida and area under curves for joint probability diagrams

Species	PAF (%)	90th centile (μg/kg wet weight)	Area under the joint probability curve (mean risk %)
All species	9.9	30.4	4.16
Flagfish	18.0	67.2	6.83
Jewel cichlid	1.9	4.2	0.88
Mayan cichlid	10.8	33.9	4.52
Mosquitofish	11.9	38.6	4.95
Marsh killifish	14.1	48.4	6.13
Sunfish	3.0	7.1	1.35

Table 9 Potentially affected fraction (PAF) of log-logistically distributed lethal body burdens of fish species from the estimated 90th centile of total endosulfan fish tissue concentrations (μg/kg wet weight) measured in fish tissue at various locations in South Florida and area under curves for joint probability diagrams

Species	PAF (%)	90th centile (μg/kg wet weight)	Area under the joint probability curve (mean risk %)
All freshwater sites	9.9	30.4	4.16
C-111	2.4	5.5	1.16
Eastern boundary	16.1	57.5	6.80
Shark River Slough	1.5	3.3	0.71
Taylor Slough	12.2	39.8	4.97

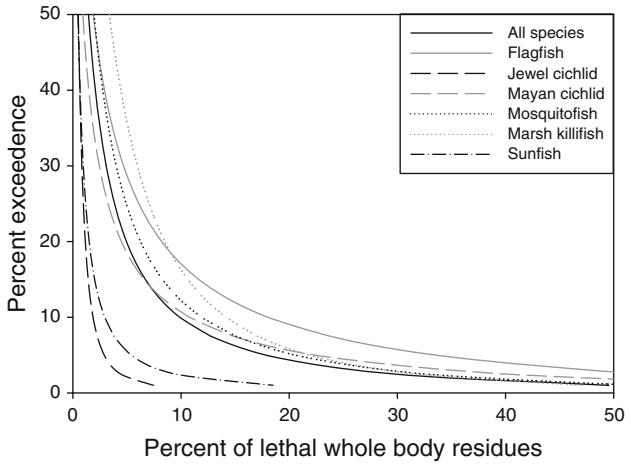

Fig. 4 Joint probability curves for the exceedence of log-logistically distributed whole body lethal doses by measured tissue concentrations of total endosulfan ($\alpha + \beta$+sulfate) in South Florida freshwater fish species

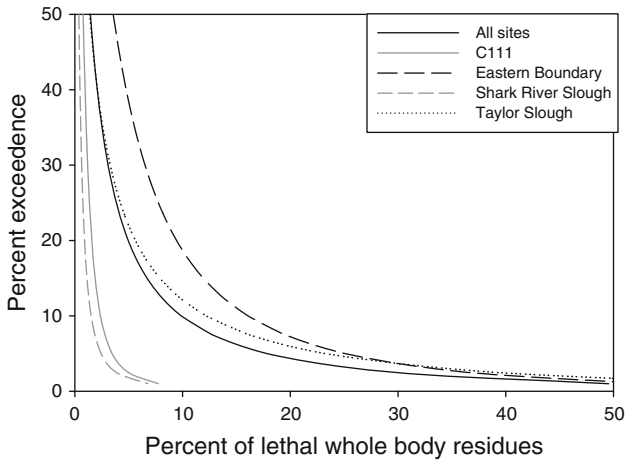

Fig. 5 Joint probability curves for the exceedence of log-logistically distributed whole body lethal doses by measured concentrations of total endosulfan ($\alpha + \beta$+sulfate) in South Florida geographic locations

of South Florida, which covers 16 counties and over 44,000 square kilometers (roughly 31% of the entire state), with over 2250 km of freshwater canals and levees, characterization of surface water exposures of α-endosulfan, β-endosulfan and endosulfan sulfate, both spatially and temporally have limitations. Many of the agricultural application locations using endosulfan are adjacent to various freshwater ecosystems, including Lake Okeechobee, the Caloosahatchee River, Kissimmee River and the St. Lucie River systems, which all contain diverse and sensitive invertebrate and fish populations, yet there are few detections of α-endosulfan, β-endosulfan and endosulfan sulfate in surface water and/or sediment in these systems. Targeted sampling for endosulfan analytical data combined with knowledge of endosulfan application locations, frequency and rates would be helpful in uncovering the exposure dynamics of endosulfan in more sensitive regions of South Florida.

Differences were found between exposures in geographic locations from surface water data and fish tissue samples. Monitoring data at S-178 in the C-111 region had the highest total endosulfan concentrations for surface water while the C-111 fish tissue samples were generally lower than sites to the north (the eastern boundary of the ENP). However, data from Loveland Slough or S-178 were limited in the database for concentrations in fish tissue. The differences between relative concentrations in site locations between surface water and fish tissue samples are likely dominated by the sampling design and spatial comparisons should take this into account.

Although the msPAF approach considers the potential risk (exceedence of toxicity values) from chemical mixtures, the probability of the mixture's components co-occurring was not explicitly addressed. The 90th centile concentration was assumed to be a suitable representation of acute exposures at a site. Therefore, applying the 90th centile concentrations to the msPAF approach assumed a co-occurrence of the mixture's components that might or might not be realistic. However, given that the 90th centile concentration estimate represents a relatively high exposure level in a distribution, its usage in the msPAF model is conservative, from a risk-based perspective, as it is more unlikely that all components would be at such a high concentration individually or together at a site.

Effects analysis Most of the laboratory aquatic toxicity data are for technical grade endosulfan with emphasis on acute effects (e.g., LC50s, EC50s). There is little chronic toxicity data for technical grade endosulfan and therefore NOEC values from chronic toxicity tests of all species were combined into one chronic SSD. Furthermore, there is very limited acute and chronic aquatic toxicity data for the α- and β-isomers and endosulfan sulfate. Aside from technical grade endosulfan, SSDs could only be developed, with limited data, for endosulfan sulfate.

Although our laboratory has completed acute toxicity studies with native fish exposed to endosulfan sulfate (Carriger et al. in preparation), the toxicity database is limited on the effects of endosulfan sulfate in water and sediment for native species in Florida.

Effects data distributions (SSDs) for acute toxicity were developed with LC50s and EC50s from the published literature. LOECs and NOECs from acute toxicity tests with endosulfan should have been used to develop SSDs to determine the extent of exceedences of these toxicity values by concentrations in surface waters to better understand the risk. Obviously, partial mortalities do occur in acute toxicity tests at concentrations below the LC50s and EC50s. However, documentation of LOECs and NOECs from acute toxicity studies in the published literature is limited for endosulfan.

Risk characterization Summarizing potential risks to toxicity endpoints from exceedences by distributions of exposure can be problematic without considering the data sources (Verdonck et al. 2003). In the current assessment, surface water exposure distributions were assembled for each site based on measured concentrations over time. Thus, the temporal variations in exposure magnitudes were examined in this approach and the resulting variance of the exposure distributions would take this into consideration assuming the distributions are robust and representative (Verdonck et al. 2003). The many sites that had low surface water detections of endosulfan increased uncertainties about risk factors derived from estimated points on the exposure distribution below the detected, plotted values. The low number of detections at all surface water sites, with the exception of S-178, adds to the uncertainty of the resulting risk predictions (PAFs and JPCs) for chronic toxicity and the complete JPCs for any surface water scenarios. For fish tissue, detections were more frequent, and the exposure distributions better represent the availability of endosulfan to fish than surface water monitoring. However, while the surface water distributions of exposure represented the temporal variation in endosulfan on a site-basis, the fish tissue distributions represented the variation within geographical regions from several sites or within groupings of fish species in sampled regions of South Florida. Thus, interpretations of risks from these distributions are based more on regional and intra-species variability in available endosulfan sampling data and not variations in time (Verdonck et al. 2003). This would affect the acceptability of exceedence statistics for risk management purposes where a temporal scenario at a site might be more acceptable than one for a region or vice versa (Verdonck et al. 2003).

Comparison of surface water exposure distributions of endosulfan (i.e., total-, $\alpha + \beta$- and endosulfan sulfate) at sampling sites to distributions of LOECs from acute toxicity studies most likely would increase the estimate of risk at surface water sampling sites. Furthermore, exceedences of NOEC distributions may have been a better measure of risk. This is especially relevant, since the Florida Fish and Wildlife Conservation Commission has a number of small-size, freshwater fish that are classified as endangered, threatened or species of special concern. Their sensitivity to endosulfan should be considered for an ecological risk assessment of endosulfan in South Florida surface waters to be complete. The limitations of using NOECs as risk values have been discussed (e.g., Chapman et al. 1996; Moore and Caux 1997; Crane and Newman 2000; Hanson and Solomon 2002) while problems with using LC50s for predicting ecological risks have also been discussed (e.g., Newman 1995; Zhao and Newman 2004; Newman et al. 2006).

This paper used existing aquatic toxicity data and ecological risk assessment methods to assess risk of measured concentrations of endosulfan but the authors acknowledge the limits of using historical water-effect and body residue-effect values for extrapolating to real-world scenarios. Area under the curve and PAF statistics cannot be used alone to represent the acceptability of risk for a scenario without considering the nature of the exposure distributions as discussed above (Verdonck et al. 2003). We also did not normalize for lipid content for tissue concentrations of endosulfan. In the Matthiessen et al. (1982) study, percent lipids for fish ranged from approximately 1–4%. The lipid data for a subset of the South Florida fish ranged from 1 to 11%, with an average of 5% (Gardinali et al. in preparation). This indicates that fish sampled in South Florida might be more likely to sequester contaminants in fat tissue and prevent interaction with chemical receptors that could produce toxic effects (Meador 2006). The approach in this study was conservative in comparisons with respect to selecting wet weight tissue concentrations for effects and exposure.

Conclusions

A probabilistic aquatic ecological risk assessment was conducted for endosulfan, the only remaining

organochlorine insecticide registered by the U.S.EPA under FIFRA in the U.S. The compound consists of two isomers (α-, β-) and the main environmental metabolite in water, sediment and tissue is endosulfan sulfate through oxidation reactions. The assessment concentrated on surface freshwater exposures of endosulfan in South Florida ecosystems in a two-tier (tier 1-hazard assessment; tier 2-probabilistic risk assessment) approach which followed the U.S.EPA framework and consisted of three phases: problem formulation, risk (exposure and effects) analysis and risk characterization. We focused on the acute and chronic risks of endosulfan by comparing distributions of surface water exposure concentrations of endosulfan [i.e., for total endosulfan (i.e., summation of concentrations of α- and β-isomers plus the sulfate), α- plus β-endosulfan, and endosulfan sulfate (alone)] with distributions of species effects toxicity data. The exceedence of the effects distribution by the exposure distribution was used as a measure of risk. Distributions of measured concentrations of total endosulfan and $\alpha + \beta$-endosulfan were compared to distributions of toxicity for technical endosulfan and distributions of endosulfan sulfate measured concentrations were compared to the distribution of toxicity for endosulfan sulfate.

The SFWMD surface water monitoring database from 1992 to 2007 was used for the concentrations of endosulfan historically found in surface waters. The distribution for endosulfan in fish tissue (whole body) from South Florida freshwaters was also used to determine the probability of exceeding critical whole body residues of endosulfan producing mortality (critical lethal residues).

Tier 1 compared actual measured concentrations in surface freshwaters in South Florida to U.S.EPA numerical WQC and found that the majority of water quality exceedences were at locations (S-177, S-178) in the C-111 system. Nine surface water sampling sites were chosen for Tier 2-probabilistic analyses. Exposure analyses showed that the highest 90th centile concentration for total endosulfan, α- + β-endosulfan and endosulan sulfate (alone) were all found at S-178 followed by S-177. At S-178 endosulfan sulfate was found in more than 60% of the surface water samples. Furthermore, the highest 90th centile concentration of endosulfan in tissue was found in flagfish (67.2 μg/kg). The whole-body residue data from the literature indicate lethal doses of endosulfan in fish tissue as low as 31 μg/kg (ww). The eastern boundary of the ENP had the highest 90th centile (57.5 μg/kg) tissue concentration out of all locations sampled. The major endosulfan constituent that was analyzed and detected in fish tissue was the metabolite endosulfan sulfate.

The aquatic toxicity database is mainly limited to technical grade endosulfan, which is acutely toxic in water to both fish (10th centile = 0.25 μg/L; 5th centile = 0.12 μg/L) and arthropods (10th centile = 0.49 μg/L; 5th centile = 0.13 μg/L). The chronic toxicity data for technical grade endosulfan are limited but it does indicate potential chronic toxicity to aquatic organisms. The published literature confirms chronic effects of endosulfan, as a result of limited short-term exposures (<96 h), at environmentally realistic surface water concentrations to fish (≤1.0 μg/L). The limited toxicity data for the isomers and endosulfan sulfate also indicate they are acutely toxic to aquatic organisms in surface water (low μg/L range). Toxicity data are also limited for exposures to the isomers and endosulfan sulfate via sediment. Aquatic toxicity data are also limited on exposures to the isomers and endosulfan sulfate in sediment.

The highest potential exceedences of the 5th or 10th centiles of the acute SSDs by surface water exposure distributions of total endosulfan were at S-178, where the 90th centile concentration for total endosulfan exceeded 12.5% (PAF) of the acute freshwater fish toxicity values. For arthropods, at the same site, the estimated 90th centile concentration for total endosulfan exceeded 7.9% (PAF) of the acute freshwater arthropod toxicity values. In Carriger et al. (2006), the probability of exceeding the estimated 10th centile concentration for chronic arthropods and predicted porewater exposures was 41%. The greater potential risk from sediment porewater could be a result of lower effect values used (chronic vs. acute) for arthropods in that assessment. The corresponding exceedence of the acute 10th centile concentration for arthropods by porewater exposures in the same site was 1% (Carriger et al. 2006). At all other freshwater sites there were less than 5% (PAF) exceedence of freshwater acute toxicity values by the 90th centile exposure concentration. Potential chronic risk (PAF of 9.2%) was only found at S-178 for total endosulfan and all other sites were <5%. In general, the risk from endosulfan sulfate is low because of the steep acute toxicity (mortality) distribution for the sulfate and also because of the minimal exceedence of the effects distribution.

Joint probability curves are presented showing the higher probability of risk at S-178 than at S-177. Freshwater fish species which contained tissue concentrations of endosulfan, likely to exceed 10% of the lethal doses, more than 10% of the time, were marsh killifish, mosquitofish and flagfish. Populations of these small demersal fish support higher trophic level (HTL) species, such as wading birds in South Florida. Declines in the abundance of HTL species, that rely upon these forage fish, have already been linked to food stress (Ley et al. 1994; Lorenz and Serafy 2006).

Based on measured exposures in surface water and available toxicity data for aquatic organisms, the PRA shows there are localized sites in South Florida where there are potential acute and chronic risks of endosulfan (total) to freshwater organisms. Furthermore, although there are always uncertainties in exposure and effects assessment,

within the ecological risk assessment process, the presence of tissue concentrations of endosulfan in small fish, at locations near and removed from ongoing agriculture activities, are findings of ecological significance. These findings become environmentally relevant, in lieu of the large number of reported aquatic incidents for fish mortality, received by the U.S.EPA in the last 30 plus years and the detection of endosulfan in biota living in distant locations, including the Arctic regions.

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