Characterization of selected organo-nitrogen herbicides in south florida canals: Exposure and risk assessments

P. Chris Wilson [a,*], Brian J. Boman [b]

[a] University of Florida/IFAS Indian River Research and Education Center (Soil and Water Science Department), 2199 South Rock Road, Fort Pierce, FL 34945, United States
[b] University of Florida/IFAS Indian River Research and Education Center (Agricultural and Biological Engineering Department), 2199 South Rock Road, Fort Pierce, FL 34945, United States

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ABSTRACT

Much uncertainty exists regarding the discharge characteristics of terrestrial-use herbicides into aquatic systems. This study evaluated the temporal distribution and concentrations of five commonly used herbicides (atrazine, bromacil, metolachlor, norflurazon, and simazine) in a typical South Florida watershed. Surface water samples were collected weekly over a 3-yr period from four canals and Ten Mile Creek. These systems received drainage water from a variety of land-uses, including residential, pastures, and citrus production. Herbicides were extracted and analyzed by GC-MS/SIM. Atrazine was most frequently detected (87% of samples) in the canal serving the residentially developed sub-basin, with median and maximum concentrations of 0.43 and 6.67 µg L^{-1}, respectively. Norflurazon was most frequently detected (90–95% of samples) in the systems serving agricultural production areas, with median and maximum concentrations ranging from 0.37–0.63 µg L^{-1} and 1.98–6.97 µg L^{-1}, respectively. Bromacil was detected in 14–36% of samples with median and maximum concentrations ranging from 0.50–0.67 µg L^{-1} and 2.31–4.96 µg L^{-1}, respectively. Metolachlor was detected in 1.8–10% of the samples, with median and maximum concentrations ranging from 0.16–0.2 µg L^{-1} and 0.17–1.55 µg L^{-1}, respectively. Simazine was detected in 10–35% of the samples, with median and maximum concentrations ranging from 0.18–0.28 µg L^{-1} and 0.37–1.35 µg L^{-1}, respectively. Bromacil + norflurazon was the most commonly detected (240 samples of 1060 total) binary combination of herbicides; whereas bromacil + norflurazon + simazine was the most frequently detected tertiary combination (58 samples). While detectable concentrations were present for significant periods of time, risks of acute toxicity were relatively low; affecting <1% of the potentially affected fraction (PAF) of plant species based on 90th centile exposure concentrations and 10th centile effects concentrations from species sensitivity distributions.

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1. Introduction

Pesticides are used throughout the world for agricultural and non-agricultural purposes. Depending on individual pesticide properties and environmental conditions, these pesticides may move from the sites of application into nearby aquatic systems. Losses of herbicides from applications in the surrounding landscape are especially important for aquatic plant communities since they are designed to control plants. Aquatic macrophytes and algae provide habitat structure and food for fish, invertebrates, water fowl, and other aquatic animals. Given their position within the landscape, discharge of herbicides in surface runoff and drainage water from surrounding land uses have potential to impact normal growth, reproduction, and health if exposure concentrations are high enough and exposure durations are long enough. Many of the streams, rivers, and lakes where these aquatic plant species occur are surrounded by land-uses that employ herbicides

for controlling weeds, or that receive runoff/drainage water from those areas.

Several commonly used herbicides in agricultural and non-agricultural settings include atrazine [6-chloro-N-ethyl-N′-(1-methylethyl)-1,3,5-triazine-2,4-diamine], bromacil [5-bromo-3-sec-butyl-6-methyluracil], metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide], norflurazon [4-chloro-5-(methylamino)-2-(3-(trifluoromethyl)phenyl)-3(2H)-pyridazinone], and simazine [6-chloro-N^2,N^4-diethyl-1,3,5-triazine-2,4-diamine]. A summary of the properties of each is shown in Table 1. These herbicides have also been detected in non-target surface water bodies around the world (Gómez-Gutièrrez et al., 2006; Konstantinou et al., 2006; Cerejeira et al., 2003; Glotfelty et al., 1984; Zablotowicz et al., 2006; Coupe et al., 1998; Byer et al., 2011; Du Preez et al., 2005; Guo et al., 2007; Bocquene and Franco, 2005; Woudneh et al., 2009). Within the South Florida area, Miles and Pfeuffer (1997) and Carriger and Rand (2008a,b) reported frequent detections of atrazine, simazine, bromacil, norflurazon, and other pesticides in 72 surface water samples collected from 1991 to 1995 in drainage canals. They reported that spatial trends in pesticide detections followed use patterns, and that bromacil, norflurazon, and

Table 1
Properties of herbicides monitored in this study.
All data adapted from Vencill (2002) unless otherwise noted.

Herbicide	CAS no.	Chemical family	Molecular weight (g mol^{-1})	Water solubility (mg L^{-1} at 25 °C)	Vapor pressure (mm Hg at 25 °C)	Log K_{ow}
Atrazine	1912-24-9	s-Triazine	215.7	33	2.9×10^{-7}	2.68
Desethyl atrazine	6190-65-4	s-Triazine	187.6[a]	3200[a]	9.3×10^{-5} [a]	1.51[a]
Desisopropyl atrazine	1007-28-9	s-Triazine	173.6	670[a]	nd	1.15[a]
Bromacil	314-40-9	Substituted uracil	261.1	700-815	3.1×10^{-7}	2.11
Metolachlor		Chloroacetamide		488	2.8×10^{-5}	
Norflurazon	27314-13-2	Fluorinated pyridazinone	303.7	28	2.9×10^{-8}	2.45
Simazine	122-34-9	s-Triazine	201.7	3.5	2.2×10^{-8}	2.18

[a] Data adapted from FOOTPRINT, 2006.

simazine were detected frequently at monitoring sites near citrus groves. Atrazine was detected regularly at all of their sites.

The presence of terrestrial-use herbicides in surface water is of concern due to the possibility of negative impacts on non-target aquatic plants and animals. Atrazine, bromacil, and simazine are all photosystem II inhibitors. They block electron transport from Q_A to Q_B by binding to the Q_B-binding niche on the D1 protein of the photosystem II complex in chloroplast thylakoid membranes (Vencill, 2002). While the blockage of electron transport stops CO_2 fixation and production of ATP and $NADPH_2$, plant death usually results from lipid and protein damage caused by free-radicals generated by the inability to reoxidize Q_A (Vencill, 2002). Norflurazon blocks carotenoid biosynthesis by inhibiting the enzyme phytoene desaturase, resulting in bleaching of foliage and destruction of chlorophyll. Metolachlor inhibits biosynthesis of several plant components, including fatty acids, lipids, proteins, isoprenoids, and flavonoids (Vencill, 2002). Using archived monitoring data from the South Florida Water Management District (SFWMD) from 1999 to 2006, Schuler and Rand (2008) conducted a risk assessment for aquatic plants within southern Florida freshwater ecosystems. They reported that the risks from individual herbicides were relatively low, but risks were higher for multiple simultaneous herbicide exposures at some sites, especially if bromacil, diuron, and norflurazon were present.

This study was initiated to 1) determine the temporal distribution and concentrations of atrazine, bromacil, metolachlor, norflurazon, and simazine in water discharged from a southern Florida watershed over a three-year period, and 2) estimate acute ecological risks to aquatic plants. These herbicides were chosen because of the prevalence of their use within the watershed and their confirmed presence in samples collected for the SFWMD's pesticide monitoring program (Pfeuffer, 2009). Two common degradation products of atrazine (desethyl-atrazine [6-chloro-2-N-propan-2-yl-1,3,5-triazine-2, 4-diamine] and desisopropyl-atrazine [2-amino-4-chloro-6-ethylamino-1,3,5-triazine]) were also included as indicators of previous atrazine/simazine exposure.

2. Methods

2.1. Drainage basin descriptions

Due to the flatness of the landscape, surface water drainage throughout much of South Florida is achieved through use of man-made drainage canals that are managed by the SFWMD (West Palm Beach, FL) (Fig. 1). These canals are equipped with various types of water control structures to maintain water levels within the canals and to regulate discharges into the receiving waterbodies. Most of these canals function to 1) remove excess water from the drainage basins, 2) supply water for agricultural needs (except ELKAM), and 3) maintain water table elevations high enough to prevent salt water intrusion into the groundwater (Table 2). The canals within this sub-basin normally drain by gravity flow through the discharge structures. Each sub-basin is relatively flat, with slopes of less than 9.3 cm km^{-1}. The network of canals and the estuary drain or recharge

the groundwater depending on head differential between the canals and the water table (Janicki et al., 1999). Samples were collected at five different sites for the duration of this study (Table 2, Fig. 1). Sampling sites were located at the discharge structures for Canal-23 (C-23/S97), Canal-24 (C-24/S49), Canal-25 (C-25/S99), Ten Mile Creek (TMC), and the ELKAM canal in Port St. Lucie (Table 2). Ten Mile creek, the ELKAM, C-23, and C-24 canals all discharge into the St. Lucie estuary, which is connected to the Indian River Lagoon estuary and the Atlantic Ocean (St. Lucie Inlet). Canal-25 discharges into the Indian River Lagoon estuary, which is connected to the Atlantic Ocean through the Fort Pierce and Sebastian inlets. A description of drainage basins for each sampling site is provided in Table 2.

2.2. Sample collection

Surface water samples were collected at least once per week from May 9, 2005 through April 8, 2008. Samples were collected upstream from each structure using Environmental Protection Agency (EPA) standard operating procedures and a modified grab sampler (U.S. EPA, 2001). Samples were collected directly into amber glass bottles by submersion to a depth of 0.76 m. Water pH was measured and recorded at the time of sampling. All samples were stored on ice upon collection. All extractions were performed within 7 d of collection and analyses were carried out within 30 d.

2.3. Sample preparation and extraction

The target herbicides were extracted from water samples using a modified version of EPA Method 3535. Samples were first filtered through a 1 μm glass fiber filter (Millipore, Billerica, MA, USA). Sample pH was adjusted to 7.0 by addition of 5–50 mL sodium phosphate buffer. HyperSep C_{18} extraction columns (500 mg/25 mL; Thermo Electron Corporation, Bellefonte, PA, USA) were initially washed with 10 mL methylene chloride, followed by sequential activation using 10 mL acetone, 10 mL methanol, and finally 10 mL reagent-grade water. Following extraction, pesticides were eluted from the columns sequentially using two 6 mL aliquots of acetone followed by two 6 ml aliquots of methylene chloride. To facilitate evaporation, the combined acetone:methylene chloride extract was first chemically dried by addition of sodium sulfate, followed by evaporation to dryness using a Labconco RapidVap system (Labconco Corp., Kansas City, MO, USA). The extracted analytes were re-dissolved in 1-mL of pesticide grade acetone. The extracts were stored at −16 °C until analyzed.

2.4. Analysis

2.4.1. Identification and quantification

Analytes were identified and quantified using an Agilent 6890 N gas chromatograph equipped with a 5975 mass spectrometer (GC-MS) (Agilent Technologies, Santa Clara, CA). The GC conditions included: inlet temperature 250 °C, transfer line 280 °C, and oven temperatures of 50 °C initial, increasing to 320 °C at a rate of 15 °C/min. with a final hold time of 0.5 min. The helium carrier gas flow rate was maintained
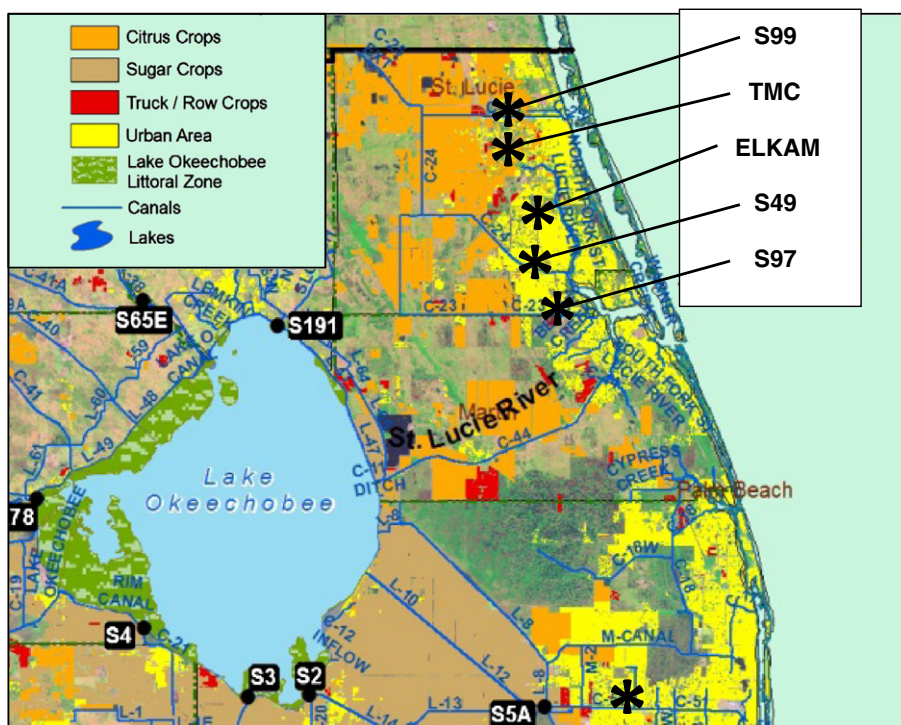
Fig. 1. Sampling site locations and surrounding land uses. Notes: land use data is for 2004; original map created 8/25/2008, edited by NC 7/20/2011. Courtesy of the South Florida Water Management District.

at 1.3 mL/min. Injections were made in splitless mode (1 µL) onto an HP-5 capillary column (30 m length, 250 µm diameter, 0.25 µm film thickness, Agilent Technologies, Santa Clara, CA). QA/QC for preparation and analysis of each sample set included method blanks, lab duplicates, field duplicates from one site, periodic field blanks/equipment blanks, surrogates, and check standards. Analytical conditions and acceptability criteria followed EPA Method 8270 C recommendations (U.S. EPA,

1996). Pesticides were quantified in single ion monitoring mode using the selected ions listed in Table 3. Quantification was based on the primary ion response if the other qualifier ion(s) were present at the correct ratios. The method detection limits/practical quantitation limits for each analyte were: 0.050/0.099 µg/L (atrazine), 0.072/0.140 µg/L (desisopropyl atrazine), 0.031/0.062 µg/L (desethyl atrazine), 0.084/0.170 µg/L (bromacil), 0.054/0.110 µg/L (metolachlor), 0.038/0.076 µg/L (norflurazon), and 0.026/0.052 µg/L (simazine). Typical recoveries were 62–68% (atrazine, metolachlor, simazine), 92% (bromacil), 40% (desethyl atrazine), 58% (desisopropyl atrazine), and 127% (norflurazon).

2.4.2. Data analysis

Summary statistics were calculated to describe the concentration of herbicides during the monitoring period. These statistics included: total number of samples, number of detections, median, minimum, and maximum concentrations. In addition, the 90th and 95th centile concentrations were calculated for each period. Centiles are useful for describing the occurrence of values below or above a given target level (e.g. if the 90th centile concentration is 0.1 mg·L^{-1}, then 90% of the values were equal to or less than 0.1 mg·L^{-1} and 10% were equal to or greater than 0.1 mg·L^{-1}). The 90th centile is commonly used to characterize exposures in ecological risk assessments (Solomon et al., 1996).

Table 2
Description of the sampling sites and respective drainage basins.

Site	Tributary	Basin area (ha)	Description
TMC (Gordy Rd.)	Ten Mile Creek	26,305	Network of canals spaced at 0.8 km (0.5 mile) intervals. Citrus production was the dominant land-use [17]. Drains successively into the North Fork of the St. Lucie River, St. Lucie Estuary, Indian River Lagoon.
S49	Canal-24 (C24)	41,506	The C24 basin is divided into 22 secondary basins, which drain into C24 through 77 outfall structures along the canal. Dominant land-uses were improved pasture and citrus production. Some single family housing development was present near the structure (Janicki et al., 1999). Drains into the St. Lucie Estuary and Indian River Lagoon.
S97	Canal-23 (C23)	303,028	C23 bisects the drainage basin. Dominant land uses within this basin were citrus production and pasture management, with concentrated urban development close to the structure (Janicki et al., 1999). Drains into the St. Lucie Estuary and Indian River Lagoon.
S99	Canal-25 (C25)	43,252	Citrus production and improved pasture management were the primary land-uses within this basin. (Pribble et al., 1999). Drains into the Indian River Lagoon.
ELKAM	ELKAM	1647	Single family residential (0.1-0.2 ha lots) is the primary land-use within the basin. Most drainage occurs through swales and secondary canals. Drains into the St. Lucie Estuary.

Table 3
Herbicide ion fragments monitored for identification and quantification by gc-ms-sim. m/z 1 used for quantification, all ions were used for identification.

Compound	m/z 1	m/z 2	m/z 3
Atrazine	200	173	
Desisopropyl Atrazine	173	158	
Desethyl Atrazine	172	174	145
Bromacil	205	207	206
Metolachlor	162	238	
Norflurazon	303	302	
Simazine	201	186	
Triphenyl phosphate	326	325	

2.4.3. Risk analysis

Risks were characterized using distributions of species-sensitivity (SSD) and exposures (Solomon et al., 1996), and the potentially affected fraction (PAF) calculation method as described by Traas et al. (2002). Freshwater aquatic plants living in the canals and connected waterways were chosen as the most sensitive organism group since they all have known target sites-of-action for the herbicides. Briefly, SSDs were constructed for each herbicide to characterize potential acute toxicity to vascular and non-vascular aquatic plants using EC50 values reported in the literature and the U.S. EPA ECOTOX database (U.S. EPA, 2007). Growth-related ecotoxicity endpoints selected included population, growth rate, abundance, biomass, photosynthesis rates, and chlorophyll. Species were only represented once in each SSD. When multiple EC50 values were available for a species, the most sensitive measurement was selected. Only EC50 values from studies reporting measured or nominal concentrations were included in the SSDs. Test durations ranged from 1 to 15 d for most studies. Algal-based growth rate studies were generally shorter in duration, while macrophyte growth rate and biomass studies were longer.

The sensitivity distributions were constructed by first ranking the EC50 values from lowest to highest, and then calculating the cumulative probability using the following equation:

$$\text{Cumulative probability} = n/[N+1] \cdot 100 \qquad (1)$$

where, n is the rank number of the datum point and N is the total number of points in the data set (Solomon et al., 1996; Schuler and Rand, 2008). The EC_{50} values were then plotted against the cumulative probability values.

Herbicide exposure distributions were constructed using the same methodology as for the species sensitivity distributions. However, in this case the concentrations detected were ranked from lowest to highest. Non-detectable concentrations were included in the distribution as zero values.

The species sensitivity distributions were plotted with the exposure distributions to graphically illustrate potential risks or margins-of-safety (MOS) (Solomon et al., 1996). Margins-of-safety were calculated using the 5th and 10th centile effects concentrations (Table 4) from the SSDs and the 90th and 95th centile exposure concentrations (Table 5), respectively. These exposure-effects scenarios represent a 1% (90th$_{exposure}$/10th$_{effect}$ centiles) and 0.25% (90th$_{exposure}$/10th$_{effect}$ centile) risk that the most sensitive species may be exposed to concentrations equal to or greater than their individual EC50's (Solomon et al., 1996). The MOS indicates how much lower the exposure concentration is relative to the target sensitivity concentration. It was calculated as:

$$\text{MOS}_x = [Y - \text{centile SSD}]/[Z - \text{centile exposure}] \qquad (2)$$

The potentially affected fraction (PAF) of plant species was estimated for each site on each sampling date for exposures to single and multiple herbicides using the methods of Traas et al. (2002) and Schuler and Rand (2008). When only one herbicide was detected in a sample, the PAF was calculated as:

$$\text{PAF} = 1 / \left(1 + e^{-(x-\alpha)/\beta}\right) \qquad (3)$$

Where,

α the mean of the log-transformed EC50 data,
x log-transformed concentration detected in the monitoring study and
β $((\sigma \cdot 3^{1/2}) \cdot \pi) \cdot 100$ (σ = standard deviation of the log-transformed data).

The calculated values were multiplied by 100 to express the PAF as a percentage. Values for α and β are shown in Table 4.

On days when more than one herbicide (except for metolachlor) was detected in a sample from a given site, the PAF was estimated using a concentration addition (CA) model as described in Schuler and Rand (2008) and Traas et al. (2002). Since the concentration addition model requires that chemicals have the same mode of action (Traas et al., 2002), metolachlor was not included in this analysis. Briefly, each EC50 concentration for each individual herbicide was divided by the estimated EC50 for the respective species sensitivity distribution. This scaling procedure transforms the exposure data into a unitless hazard unit (HU), which is analogous to the toxic unit. Following the HU transformation, all of the HU-EC50 data for each herbicide were combined, ranked from lowest to highest HU, and the β coefficient was estimated (as previously described) to be 0.36. The HU's from co-detected herbicides were summed on each day to calculate the cumulative HU due to all of the herbicides in the sample. The HU and β estimates were then applied to the following equation to estimate PAF due to multiple herbicides (PAF$_{mh}$) (Traas et al., 2002):

$$\text{PAF}_{mh} = 1 / \left[1 + e^{-\log(\Sigma HU)/\beta}\right] \cdot 100 \qquad (4)$$

The calculated PAF$_{mf}$ values were multiplied by 100 for expression as percentages.

3. Results

3.1. Concentrations and frequency characterization

Detectable concentrations of atrazine were most common in water samples collected from the ELKAM, S97, and S49 sampling sites, being present in 87, 46, and 25% of the 211–212 samples collected, respectively (Table 5, Fig. 2A, A1). Minimum/maximum/median concentrations were 0.13/0.67/0.43 µg/L (ELKAM); 0.09/10.10/0.28 µg/L (S49); and 0.11/1.19/0.34 µg/L (S97). Unfortunately, it was not possible to measure actual flow at the ELKAM site to correlate concentrations of atrazine. However, based on field notes water was almost always flowing at the site. Concentrations of atrazine tended to be higher at the S97 and S49 sites when flow was lower, reflecting reduced dilution due to watershed drainage into the canals (A1). Atrazine was detected in 4 of the 213 samples collected at the Ten Mile Creek site, and in 5 of the 212 samples collected at the S99 site. Minimum/maximum/median concentrations were 0.15/0.24/0.19 µg/L (TMC) and 0.11/1.19/0.34 µg/L (S97). Median concentrations were generally lower than mean concentrations at all sites, indicating that the concentration distributions were skewed towards lower concentrations.

Two degradation products of atrazine, desethyl atrazine and desisopropyl atrazine, were also detected at some sites. Desethyl atrazine was detected most frequently in samples from the ELKAM (Table 5, A2). In this case it was detected in 36% of the samples with concentrations ranging from 0.04 to 0.59 µg/L. The median detectable concentration

Table 4
Parameters of the logistic distribution fitted to the log of acute toxicity values for herbicides to aquatic algae and plants.

Compound	n	R^2	α[a]	β[b]	Centile[c]	
					5th	10th
Atrazine	24	0.98	2.03	0.33	14.1	21.2
Desisopropyl Atrazine[d]	--	–	–	–	–	–
Desethyl Atrazine[d]	–	–	–	–	–	–
Bromacil	7	0.95	1.58	0.42	1.2	2.7
Metolachlor	21	0.97	2.44	0.40	13.1	23.2
Norflurazon	9	0.93	1.82	0.28	4.3	9.7
Simazine	17	0.95	1.98	0.39	5.0	9.5

[a] Mean of the log-transformed toxicity values.
[b] Scale parameter (Schuler and Rand, 2008).
[c] µg L^{-1}.
[d] Data not available.

Table 5

Summary of detectable herbicide concentrations ($\mu g\ L^{-1}$) at the ELKAM, Ten Mile Creek (TMC), C-23 (S97), C-24 (S49), and C-25 (S99) sampling sites. $N = 212$ for the ELKAM, S-49, and S-99 sites. $N = 213$ for Ten Mile creek, and $N = 211$ for S-97.

Site	Summary statistics				Centile		Margin-of-safety	
	Detections	Median	Min	Max	90th	95th	MOS$_{10}$[a]	MOS$_5$[b]
Atrazine								
ELKAM	185	0.43	0.13	6.67	0.922	1.719	23.1	8.2
TMC	4	0.19	0.15	0.24	–[c]	–[c]	–[c]	–[c]
S49	98	0.28	0.09	10.10	0.605	0.643	35.0	21.9
S97	54	0.34	0.11	1.19	0.535	0.592	39.6	23.8
S-99	5	0.18	0.14	0.46	–[c]	–[c]	–[c]	–[c]
Desethyl-atrazine								
ELKAM	76	0.27	0.04	0.59	0.280	0.316	–[c]	–[c]
TMC	4	0.26	0.22	0.27	–[c]	–[c]	–[c]	–[c]
S49	12	0.23	0.05	0.47	–[c]	0.096	–[c]	–[c]
S97	1	1.19	1.19	1.19	–[c]	–[c]	–[c]	–[c]
S-99	2	0.17	0.12	0.22	–[c]	–[c]	–[c]	–[c]
Desisopropyl-atrazine								
ELKAM	3	0.1	0.07	0.41	–[c]	–[c]	–[c]	–[c]
TMC	4	0.34	0.14	0.42	–[c]	–[c]	–[c]	–[c]
S49	6	0.19	0.13	0.42	–[c]	–[c]	–[c]	–[c]
S97	4	0.29	0.15	0.71	–[c]	–[c]	–[c]	–[c]
S-99	2	0.42	0.41	0.43	–[c]	–[c]	–[c]	–[c]
Bromacil								
ELKAM	31	0.50	0.23	2.33	0.421	0.564	6.4	2.1
TMC	51	0.57	0.14	4.59	0.621	0.870	4.3	1.4
S49	70	0.61	0.19	3.78	0.865	1.40	3.1	0.9
S97	52	0.56	0.16	2.31	0.656	0.982	4.1	1.2
S-99	77	0.67	0.20	4.96	0.808	1.448	3.3	0.8
Metolachlor								
ELKAM	4	0.16	0.11	0.17	–[c]	–[c]	–[c]	–[c]
TMC	9	0.19	0.06	0.29	–[c]	–[c]	–[c]	–[c]
S49	21	0.17	0.09	1.44	–[c]	0.161	–[c]	81.4
S97	22	0.20	0.12	1.55	0.120	0.205	193.3	63.9
S-99	9	0.17	0.16	0.29	–[c]	–[c]	–[c]	–[c]
Norflurazon								
ELKAM	9	0.34	0.18	0.74	–[c]	–[c]	–[c]	–[c]
TMC	203	0.47	0.11	2.69	1.120	1.482	20.7	8.8
S49	194	0.37	0.08	1.98	0.898	1.000	25.8	13.1
S97	196	0.39	0.06	6.97	0.922	1.057	25.1	12.3
S-99	191	0.63	0.01	3.18	1.277	1.662	18.1	7.9
Simazine								
ELKAM	17	0.28	0.04	0.59	–[c]	0.247	–[c]	20.2
TMC	33	0.28	0.07	0.96	0.277	0.312	34.2	16.0
S49	22	0.18	0.06	0.37	0.054	0.184	175.9	27.1
S97	32	0.25	0.09	0.83	0.177	0.282	53.6	17.7
S-99	41	0.27	0.09	1.35	0.270	0.310	35.1	16.1

[a] Margin-of-safety for the 90th centile concentration and 10th centile of the species sensitivity distribution (SSD) (most sensitive species).
[b] Margin-of-safety for the 95th centile concentration and 5th centile of the SSD.
[c] No value.

was 0.27 μg/L. Desethyl-atrazine was detected in 1–12 samples collected at the other four sites (Table 5, A2). The highest concentration detected was at the S97 site, where the only detectable concentration was 1.19 μg/L. Median concentrations at the other three sites ranged from 0.17 to 0.26 μg/L. Desisopropyl atrazine was only detected in 2–6 samples collected between all of the sites (Table 5, A3). Median concentrations ranged from 0.1 to 0.42 μg/L. The maximum concentration detected was 0.71 μg/L, occurring at the S97 site.

Bromacil was detected most frequently at the S49 (70) and S99 (77) sites (Table 5, Fig. 2B, A4). Median detectable concentrations at each respective location were 0.61 μg/L and 0.67 μg/L. Bromacil was detected in 31 to 51 samples collected at the other locations with median concentrations of 0.50 μg/L (ELKAM), 0.57 μg/L (TMC), and 0.56 μg/L

(S97). The highest concentrations detected were 4.59 μg/L (TMC) and 4.96 μg/L (S99).

Metolachlor was present in 21 and 22 of the samples collected at the S49 and S97 sites, respectively (Table 5, Fig. 2C, A5). Median detectable concentrations at these sites ranged from 0.17 to 0.20 μg/L. Metolachlor was only detected in 4 to 9 samples collected at the other sites, with median concentrations ranging from 0.16 to 0.19 μg/L. The two highest concentrations were 1.44 μg/L (S49) and 1.55 μg/L (S97).

Norflurazon was the most frequently detected herbicide. It was detected in 191 to 203 samples collected from the TMC, S49, S97, and S99 sites (Table 5, Fig. 2D, A6). In contrast, it was only detected in nine samples from the ELKAM site. Median detectable concentrations were 0.34 μg/L (ELKAM), 0.47 μg/L (TMC), 0.37 μg/L (S49), 0.39 μg/L (S97), and 0.63 μg/L (S99). The highest concentrations of norflurazon at each site were 0.74 μg/L (ELKAM), 2.69 μg/L (TMC), 1.98 μg/L (S49), 6.97 μg/L (S97), and 3.18 μg/L (S99).

Simazine was most frequently detected in samples from the ELKAM, where 76 of the 212 samples collected contained detectable concentrations (Table 5, Fig. 2E, A7). The median detectable concentration at the ELKAM was 0.28 μg/L. Simazine was detected in 22 to 41 of the 211–213 samples collected at the other sites, with median concentrations ranging from 0.18 to 0.28 μg/L. The maximum concentration (1.35 μg/L) was detected at S99.

3.2. Multiple herbicide detection events

The presence of multiple herbicides simultaneously can significantly modify the onset and expression of toxicity to aquatic organisms. A summary of the different combinations of co-occurring herbicides is shown in Table 6. Among the binary combinations, the atrazine + norflurazon was the most frequently detected combination at any one site, occurring in 91 samples collected at the S49 site. This combination occurred in 54 samples collected at the S97 site and 4 to 6 samples at the other sites. The bromacil + norflurazon and simazine + norflurazon combinations were the most frequently detected across all sites, occurring in 49 to 71 (BRO + NOR) and 22 to 41 (NOR + SIM) samples at all of the sites except the ELKAM. Norflurazon + metolachlor was most frequently detected at the S49 (20 samples) and S97 (22 samples) sites. Atrazine + desethyl atrazine, atrazine + simazine, and atrazine + bromacil were the most frequently detected combinations at the residential ELKAM site, occurring in 70, 15, and 23 samples, respectively.

Among the tertiary combinations, the simazine + bromacil + norflurazon combination was the most frequently detected combination detected across all sites, occurring in 12 to 18 samples, except for the ELKAM (Table 6). Samples collected from S49 had the highest frequency of detectable tertiary combinations (89 occurrences). The atrazine + simazine + norflurazon, atrazine + bromacil + norflurazon, atrazine + norflurazon + metolachlor, and simazine + bromacil + norflurazon combinations occurred in 12, 26, 15, and 13 samples, respectively at S49. The second and third highest frequency tertiary combinations occurred at S97 (59 occurrences) and S99 (49 occurrences), respectively.

3.3. Risks to aquatic plants

Risks of acute toxicity to freshwater plants due to each individual herbicide were relatively low for the majority of the 3-year monitoring period. In all cases there was a margin of safety for the 90th centile concentration detected and 10th centile for the species sensitivity distribution (Table 5). Margins-of-safety were lowest for bromacil, followed by norflurazon. Environmental concentrations ranged from 3.1–6.4 (bromacil) and 18.1–25.8 (simazine) times lower than the 10th centile for the sensitivity distribution. Margins-of-safety at the 95th centile exposure level were present for all of the herbicides except bromacil (Table 5). The 95th centile environmental concentration was greater than the 5th centile sensitivity concentration at the S49 and
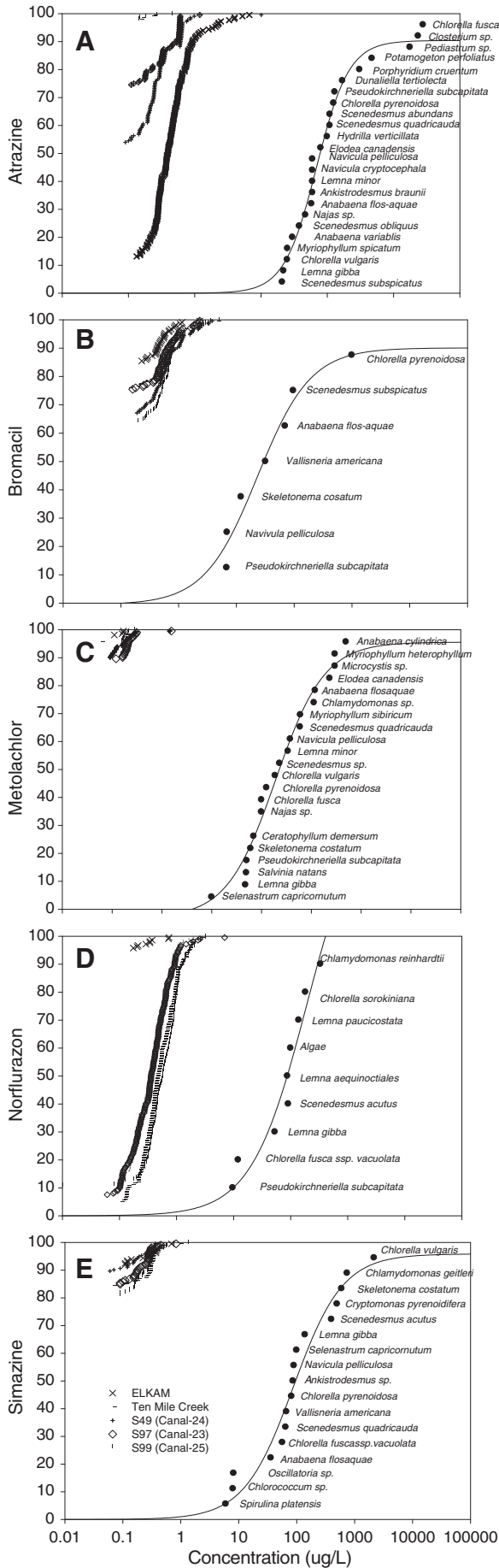
Fig. 2. Cumulative probability distributions for herbicides detected during the study, and for published aquatic algae and macrophyte species (EC50 response level).

Table 6

Detection frequency for each herbicide combination detected at water control structures for the ELKAM waterway, Ten Mile Creek (TMC), Canal-24 (S49), Canal-23 (S97), and Canal 25 (S99). Herbicide abbreviations: atrazine (ATZ), desisopropyl atrazine (DI), desethyl atrazine (DE), bromacil (BRO), metolachlor (MET), norflurazon (NOR), and simazine (SIM).

Herbicides	Site				
	ELKAM	TMC	S49	S97	S99
Binary combinations					
ATZ + DI	3	1	4	1	1
ATZ + DE	70	3	12	1	1
ATZ + SIM	15	1	12	8	3
ATZ + BRO	23	2	28	10	3
ATZ + NOR	6	4	91	54	5
ATZ + MET	3	0	16	6	3
SIM + BRO	4	12	13	14	18
SIM + NOR	2	33	22	32	41
SIM + MET	1	1	3	6	7
BRO + NOR	4	49	65	51	71
BRO + MET	3	4	7	6	6
NOR + MET	2	9	20	22	8
Tertiary combinations					
ATZ + SIM + BRO	3	1	6	3	3
ATZ + SIM + NOR	1	1	12	8	3
ATZ + SIM + MET	0	0	2	3	3
ATZ + BRO + NOR	2	2	26	10	3
ATZ + BRO + MET	2	0	5	3	3
ATZ + NOR + MET	1	0	15	6	3
SIM + BRO-NOR	1	12	13	14	18
SIM + NOR + MET	1	1	3	6	7
BRO + NOR + MET	1	4	7	6	6

S99 sites, indicating that some toxic effects could be expected for the most sensitive species. As with the 90th centile exposure protection level, margins-of-safety were smallest for bromacil and norflurazon with environmental concentrations ranging from 0.8–2.1 (bromacil) and 7.9–13.1 (simazine) times lower than the 5th centile sensitivity distribution concentration (Table 5).

The maximum potentially affected fraction (PAF) of plant species from metolachlor and simazine exposures at any of the sites were 0.3 and 0.6%, respectively (data not shown). The maximum PAFs from atrazine exposure were greatest at S49 (4.3%) and the ELKAM (2.5%) for any given sampling event. The maximum PAFs for bromacil were the greatest of all the herbicides, ranging from 5.2–10.8% across all the sites. Maximum PAFs from norflurazon ranged from 0.4–0.9% for all of the sites except the ELKAM, which was <0.1%.

In addition to evaluating the PAF from individual herbicides, the PAF from multiple herbicides was also evaluated. During the majority of the monitored period the PAF was less than 1%, with several excursions above this value (Fig. 3). The maximum PAF observed for each site on individual sampling dates were 8.1% (ELKAM), 2.5 (TMC), 13.3% (S49), 3.6% (S97), and 3.1% (S99) (Fig. 3). The PAF 90th, 95th, and 99th centiles for all of the sites were ≤0.9%, ≤1.7%, and ≤4.6% for the individual + multiple herbicide exposures (Table 7), indicating a relatively low potential for acute toxicity due to these herbicides.

4. Discussion

Herbicide detectability seemed to mirror land uses for some of the herbicides. In Florida, atrazine is used primarily for turfgrass maintenance in urban areas and for weed control in some vegetable crops. This non-restricted herbicide is readily available to homeowners. Detections of atrazine were lowest at the TMC and S99 sites, which served primarily
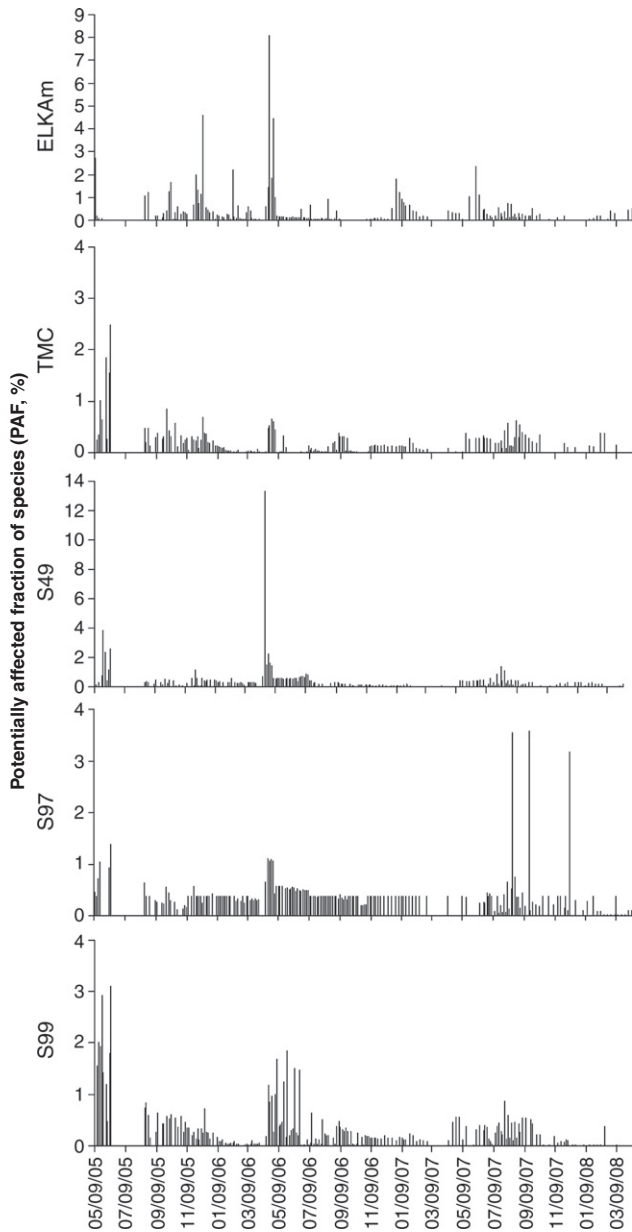
Fig. 3. Potentially affected fraction (PAF,%) of aquatic plant species impacted by all of the herbicides detected (single or mixtures when present) during the study at each sampling location on each sampling date.

citrus and pasture production land uses where atrazine is not labeled for use. Detections at the S49 and S97 sites were intermediate, possibly reflecting the intermediate degree of residential development close to the sites (Janicki et al., 1999). The drainage basins included primarily citrus and pastures further west, with residential development occurring

Table 7
Centile values (%) for the potentially affected fraction (PAF) of freshwater aquatic plant species exposed to individual and multiple herbicides detected during study.

Site	Individual and multi-herbicide PAFs (combined)		
	90th	95th	99th
S99	0.7	1.5	2.9
S97	0.6	0.9	3.6
S49	0.7	1.4	3.9
TMC	0.4	0.6	1.9
ELKAM	0.9	1.7	4.6

closer to the sampling sites. Unlike atrazine, norflurazon was dominant in water from the primarily agricultural drainage basins. Detections of simazine and bromacil were intermediate, appeared to be distributed across all sites. These herbicides have right-of-way labeled uses which may account for their more cosmopolitan presence. Metolachlor was present in more samples from the S49 and S97 sites, also with higher concentrations than the other sites. This herbicide is labeled for weed control in agricultural production, residential landscape maintenance, and highway right-of-way maintenance.

Uncertainty exists with any risk assessment. In this case, the concentrations detected during each week of sampling are assumed to be representative of the concentration present until the next sampling interval. This may be a good assumption during low-flow periods, but may be less robust during periods with high flow volumes (Wilson et al., 2007a,b; Wilson and Ferguson-Foos, 2006; Wilson et al., 2004). Furthermore, this assessment assumes that only the compounds monitored for may contribute to toxicity. However, other herbicides may have been present that were not amenable to the extraction and analysis methods used in this study. For example, Schuler and Rand (2008) identified significant risks from diuron, in addition to bromacil and norflurazon. Even though atrazine was present for the majority of the 3-yr monitoring period at the ELKAM and C23/24 sites, little acute toxicity was present based on the PAF analysis. This also agreed with the results reported by Schuler and Rand (2008).

The degree of toxic effects is also uncertain. The PAF analysis assumes that the "universe" of species may be present in the water body under investigation, and that the potentially affected fraction of all those organisms can be predicted. However, toxicity data for bromacil and norflurazon were scarce, leading to more uncertainty in the SSD model and in predicting the PAF. Additionally, the SSDs were constructed using EC50 values. As such, these values imply a reduction in the respective growth measurement of 50%. In some situations this may not be an acceptable implication. Use of NOEC or LOECs could be more protective of aquatic species, but those data were not available for all of these herbicides.

5. Conclusions

This study provided a high resolution description of the temporal distribution and concentrations of herbicides in several diverse watershed sub-basins over a 3-yr. period. Atrazine was nearly always detected in the canal within the residentially-developed drainage sub-basin, while norflurazon was nearly always detected in the canals serving agricultural land-uses. This study also identified common 2- and 3-component mixtures of herbicides present. Concentrations were generally low for all herbicides during the majority of the 3-year monitoring period. The PAFs (single and multiple herbicide exposures) for the majority of the study at all sites was less than 1%, indicating low potential for acutely toxic effects to aquatic plants.

Supplementary materials related to this article can be found online at doi:10.1016/j.scitotenv.2011.09.058.

References

Bocquene G, Franco A. Pesticide contamination of the coastline of Martinique. Mar Pollut Bull 2005;51:612–9.

Byer JD, Struger J, Sverko E, Klawunn P, Todd A. Spatial and seasonal variations in atrazine and metolachlor surface water concentrations in Ontario (Canada) using ELISA. Chemosphere 2011;82:1155–60.

Carriger JF, Rand GM. Aquatic risk assessment of pesticides in surface waters in and adjacent to the Everglades and Biscayne National Parks: I. Hazard assessment and problem formulation. Ecotoxicology 2008a;17:660–79.

Carriger JF, Rand GM. Aquatic risk assessment of pesticides in surface waters in and adjacent to the Everglades and Biscayne National Parks: II. Probabilistic analyses. Ecotoxicology 2008b;17:680–96.

Cerejeira MJ, Viana P, Batista S, Pereira T, Silva E, Valerio MJ, et al. Pesticides in Portuguese surface and ground waters. Water Res 2003;37:1055–63.

Coupe RH, Thurman EM, Zimmerman LR. Relation of the occurrence of cotton and rice herbicides in three streams of the Mississippi Delta. Environ Sci Technol 1998;32: 3673–80.

Du Preez LH, Jansen van Rensburg PJ, Jooste AM, Carr JA, Giesy JP, Gross TS, et al. Seasonal exposures to triazine and other pesticides in surface waters in the western high yield corn-production region in South Africa. Environ Pollut 2005;135:131–41.

FOOTPRINT. The FOOTPRINT pesticide properties database. Database collated by the University of Hertfordshire as part of the EU-funded FOOTPRINT project (FP6-SSP-022704); 2006. http://www.eu-footprint.org/ppdb.html.

Glotfelty DE, Taylor AW, Isensee AR, Jersey J, Glen S. Atrazine and simazine movement to Wye River Estuary. J Environ Qual 1984;13:115–21.

Gómez-Gutièrrez AI, Jover E, Bodineau L, Albaiges J, Bayona JM. Organic contaminant loads into the Western Mediterranean Sea: estimate of Ebro River inputs. Chemosphere 2006;65:224–36.

Guo L, Kelley K, Goh KS. Evaluation of sources and loadings of pesticides to the Sacramento River, California, USA, during a storm event of winter 2005. Environ Toxicol Chem 2007;26:2274–81.

Janicki A, Wade D, Pribble JR, Squires A. St. Lucie watershed assessment Task A: watershed characterization — final report. West Palm Beach, FL: South Florida Water Management District; 1999. [February].

Konstantinou IK, Hela DG, Albanis TA. The status of pesticide pollution in surface waters (rivers and lakes) of Greece: Part I. Review on occurrence and levels. Environ Pollut 2006;141:555–70.

Miles CJ, Pfeuffer RJ. Pesticides in canals of south Florida. Arch Environ Contam Toxicol 1997;32:337–45.

Pfeuffer RJ. Ambient pesticide monitoring network: 1992 to 2007. Technical Publication SFWMD 105. West Palm Beach, FL: South Florida Water Management District; 2009. p. 57.

Pribble JR, Squires A, Reiter M, Janicki A, Wade D. C-25 basin and basin 1 watershed assessment. Watershed Characterization, vol. A. West Palm Beach, FL: South Florida Water Management District; 1999.

Schuler LJ, Rand GM. Aquatic risk assessment of herbicides in freshwater ecosystems of South Florida. Arch Environ Contam Toxicol 2008;54:571–83.

Solomon KR, Baker DB, Richards RP, Dixon KR, Klaine SJ, LaPoint TW, et al. Ecological risk assessment of atrazine in North American surface waters. Environ Toxicol Chem 1996;15:31–76.

Traas TP, van de Meent D, Posthuma L, Hamers T, Kater BJ, de Zwart D, et al. The potentially affected fraction as a measure of ecological risk. In: Posthuma L, Traas T, Suter GW, editors. Species sensitivity distributions in ecotoxicology. Boca Raton, FL: Lewis Publishers; 2002. p. 285–313.

U.S. Environmental Protection Agency. ECOTOX User Guide:ECOTOXicology Database System. [Version 4.0. Available] http://www.epa.gov/ecotox/2007. [Accessed 3/9/2011].

U.S. Environmental Protection Agency (US EPA). Method 8270C, Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS), Revision 3. SW-846 Online: Test Methods for Evaluating Solid Wastes, Physical/Chemical Properties; 1996. www.epa.gov/epaoswer/hazwaste/test/main.htm.

U.S. Environmental Protection Agency (US EPA). Environmental investigations standard operating procedures and quality assurance manual. Region 4, Athens, GA; 2001.

Vencill WK. Herbicide handbook. 8th ed. Lawrence, KS: Weed Science Society of America; 2002.

Wilson PC, Ferguson-Foos J. Survey of carbamate and organophosphate pesticide export from a South Florida agricultural watershed: implications of sampling frequency on ecological risk estimation. Environ Toxicol Chem 2006;25:2847–52.

Wilson PC, Ferguson-Foos J, Jones R. Pulsed losses and degradation of aldicarb in a South Florida agricultural watershed. Arch Environ Contam Toxicol 2004;48: 24–31.

Wilson PC, Boman BJ, Ferguson-Foos J. Norflurazon and simazine losses in surface runoff water from flatwoods citrus production areas. Bull Environ Contam Toxicol 2007a;78(5):341–4.

Wilson PC, Boman BJ, Ferguson-Foos J. Non-target deposition and losses of oxamyl in surface runoff from flatwoods citrus production areas. Environ Toxicol Chem 2007b;26:201–7.

Woudneh MB, Ou Z, Sekela M, Tuominen T, Gledhill M. Pesticide multiresidues in waters of the lower Fraser Valley, British Columbia, Canada. Part I. Surface water. J Environ Qual 2009;38:940–7.

Zablotowicz RM, Locke MA, Krutz LJ, Lerch RN, Lizotte RE, Knight SS, et al. Influence of watershed system management on herbicide concentrations in Mississippi Delta oxbow lakes. Sci Total Environ 2006;370:552–60.