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FATE AND RISK OF ATRAZINE AND SULFENTRAZONE TO NONTARGET SPECIES AT AN AGRICULTURE SITE

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Abstract: The present study evaluated the risk associated with the application and co-occurrence of 2 herbicides, atrazine and sulfentrazone, applied to a 32-ha corn and soybean rotational field. Field concentrations of the compounds were measured in soil, runoff water, and groundwater, with peak mean atrazine and sulfentrazone concentrations found in the soil (144 ng/g dry wt, and 318 ng/g dry wt, respectively). Individual and mixture laboratory bioassays were conducted to determine the effects of atrazine and sulfentrazone on the survival of *Daphnia magna* and *Pimephales promelas,* the germination of *Lactuca sativa*, and the growth of *Pseudokirchneriella subcapita* and *Lemna minor*. *Pseudokirchneriella subcapita* and *L. minor* were the most susceptible species tested, and the effects on growth of the herbicides in mixtures best fit an independent action model. Risk quotients and margin of safety of 10% (MOS10) values were used to estimate risk and were calculated using runoff water concentrations. The MOS10 values were more sensitive than risk quotients in estimating risk. The MOS10 value for sulfentrazone runoff water concentration effects on *P. subcapita* was 7.8, and for *L. minor* was 1.1, with MOS10 values < 1 indicating potential risk. Overall, the environmentally relevant concentrations fell below the effect concentrations; therefore, atrazine and sulfentrazone posed little to no risk to the nontarget species tested. *Environ Toxicol Chem* 2017;36:1301–1310. © 2016 SETAC

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INTRODUCTION

In 2012, 98% of soybeans and 99% of corn grown in the United States were treated with herbicides [1]. One of the most commonly used herbicides to improve agricultural yields is atrazine, used to control broadleaf and grassy weeds [2]. In 2014, 55% of corn grown in the United States was treated with atrazine, equivalent to 2.05×10^7 kg of atrazine active ingredient (a.i.) [1]. The environmental half-life of atrazine in soil is dependent on soil type and climate and ranges from 13 d to 261 d [3]. Because it is relatively water soluble and mobile, atrazine is susceptible to leaching and runoff into nearby water bodies. It can also persist in surface waters for extended periods of time, with an environmental half-life of more than 90 d [2]. Atrazine is the most common pesticide detected in surface and groundwater in the United States, with major concentration peaks in spring and early summer [3]. As a photosystem II inhibitor, atrazine affects plant metabolic processes [4], and prolonged atrazine exposure in water can have toxic effects on nontarget aquatic species [5,7]. Median effect concentrations (EC50s) reported in the literature vary for nontarget aquatic species, with algae being the most susceptible, followed by vascular plants, invertebrates, and fishes [5–7]. In both aquatic and terrestrial vertebrates, atrazine is not typically acutely toxic [4], but some studies suggest it is an endocrine-disrupting chemical [2].

Another commonly used herbicide is the aryl triazolinone herbicide sulfentrazone. Sulfentrazone is registered for use on several agricultural crops (e.g., soybeans), ornamental turf and plant products, residential lawns, and recreational areas (e.g., golf courses) [8]. Sulfentrazone inhibits protoporphyrinogen oxidase, an important enzyme in chlorophyll biosynthesis, causing extreme dehydration of foliage and necrosis due to loss of membrane integrity [9]. Little is known about the fate and transport of sulfentrazone. The primary route for the dissipation of sulfentrazone in soil is microbial degradation, and the reported environmental half-life ranges from 110 d to 280 d depending on the soil and environmental conditions [10]. Due to the relatively long environmental half-life in soil and relatively high water solubility [8], sulfentrazone is likely to be transported into the aquatic environment. There are no studies on the effects of sulfentrazone to nontarget aquatic species other than those conducted for the US Environmental Protection Agency (USEPA) registration of sulfentrazone. These studies indicate that nontarget aquatic vascular plants are the most susceptible to sulfentrazone, followed by phytoplankton, aquatic invertebrates, and fishes [8]. As a result of the common farming practice of a corn and soybean yearly rotation, overlap of atrazine and sulfentrazone is possible in soils, surface water, and groundwater in the Midwestern United States. Although the toxicity of herbicides with other pesticide classes has been characterized [11,12], there are only a few published studies that address herbicide–herbicide interactions [5,13], and none have examined the potential mixture effects of atrazine and sulfentrazone. The abundance of atrazine and the common practice of corn soybean rotation used in Illinois (USA) [1] make it an ideal location to evaluate the potential risk associated with their overlapping concentrations.

The objective of the present study was to characterize the risk to nontarget species associated with exposure to atrazine and sulfentrazone from an agricultural field planted in corn in year 1 and soybeans in year 2. Environmental exposure potential was determined by tracking the fate of atrazine and

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sulfentrazone in a 32-ha agricultural field in Christian County, Illinois. Field concentrations of the herbicides were measured in surface soil, surface runoff water, and groundwater throughout the 2-yr study. Nontarget species sensitivity was determined using laboratory bioassays. The toxicity of atrazine and sulfentrazone to aquatic species *Daphnia magna* and *Pimephales promelas* and plant species *Pseudokirchneriella subcapita*, *Lemna minor*, and *Lactuca sativa* was evaluated individually and in combination. Risk was characterized using risk quotient and margin of safety analyses as described below.

MATERIALS AND METHODS

Field study

The agricultural field used in the present study was located in Christian County in Central Illinois. The 32-ha field utilized a no-till system with a corn and soybean yearly rotation, with the use of atrazine and sulfentrazone, respectively. The field was sampled from January 2013 to October 2014. In 2013, 2 applications of an atrazine formulation were made using a ground boom spray. The preplant application of atrazine occurred in May 2013 at a rate of 1120 g a.i./ha, and a postplant application occurred in June 2013 at a rate of 561 g a.i./ha. Two applications is the maximum number of applications suggested by most commercially available chemical labels, and a split application is fairly common [14]. After harvest in November 2013, a sulfentrazone formulation was applied to the field using ground boom spraying at 174 g a.i./ha, and an identical second application, postplanting but pre-emergent soybean, occurred 6 mo later (May 2014). Sulfentrazone was applied to control broadleaf weeds and sedge species. Other traditional crop treatments were also performed uniformly across the field throughout the growing season, including the application of anhydrous ammonia, potash, and nitrogen.

Field sample collection

Surface soil samples from the top 2 cm of soil were collected twice monthly for 3 mo after each application for herbicide analyses, then continued monthly until after harvest. Eighteen different locations were sampled (Supplemental Data, Figure S1) at the north and south sides of the field, so they would be accessible throughout the growing season. Polyvinyl chloride (PVC) pipes were cut in half lengthwise to make 6 overland runoff troughs, 2 m long, that were capped at both ends. Lack of compound binding to the troughs was confirmed prior to the field study. These troughs were used to collect runoff water at 6 locations throughout the field (Supplemental Data, Figure S1). The runoff water troughs were also divided among the north and south sides of the field for accessibility. Water samples were collected monthly, when available, following a natural rain event equal to or greater than 1.27 cm in a period of less than 24 h. Six groundwater wells (~2.5 m deep) were also installed in the field prior to the beginning of the present study, and water was collected monthly from the wells when available.

Soil samples were collected using a metal hand trowel, runoff samples were collected using an aluminum scoop, and groundwater samples were collected using an electric pump powered by a 12-V lawnmower battery. All samples were transferred into light-restrictive glass jars previously rinsed with pesticide grade acetone. The samples were transported back to the laboratory at Southern Illinois University in coolers and stored in the dark at 4 °C until the time of extraction. Maximum holding time was 30 d for soil samples and 14 d for the water samples, to prevent degradation of the herbicides in the environmental samples [15].

Solvents and chemicals

Atrazine and sulfentrazone were obtained from ChemService and were certified by the suppliers to have >98% purity. The surrogate, propanil, was purchased from ChemService and had a certified purity of 99.5%. All other solvents and materials were purchased from Fisher Scientific.

Soil and water extractions

Soil extraction procedures from Ohmes and Mueller [16] were slightly modified for the present study. Field soil was air dried in the dark overnight, ground using a mortar and pestle, and then sieved (2 mm) to remove plant material. A portion of the soil (20 g) was spiked with 400 ng of propanil surrogate. The soil was extracted using methanol (40 mL) by rotating for 16 h at 180 rpm on a MaxQ 200 shaker table (Thermo Scientific), and methanol (40 mL) was added to extract the analytes. A portion of the supernatant (5 mL) was evaporated to near dryness using a Pierce Reactivap Model 1878, solvent changed to acetone, filtered with a 0.45 μm Whatman filter vial, and reconstituted with 500 μL of mobile phase (90% acetonitrile and 10% high performance liquid chromatography [HPLC]-grade water acidified with 0.1% phosphoric acid) to be analyzed on the HPLC.

Prior to extracting atrazine and sulfentrazone from field-collected water, each sample (250 mL) was filtered through a 0.63-μm sieve to remove plant debris and transferred into a 600-mL beaker. The water sample was spiked with 100 ng of propanil surrogate stock. The water sample was then extracted using a C8 solid phase extraction cartridge (Hypersep 500 mg; Dionex) that was primed with acetone (4 mL) followed by deionized water (4 mL) on a vacuum manifold (Supelco; Sigma–Aldrich). The compounds were then eluted from the cartridge with 10 mL of pesticide grade acetone, concentrated under a gentle stream of nitrogen on a Reactivap and reconstituted with 500 μL of mobile phase solution (90% acetonitrile and 10% HPLC-grade water acidified with 0.1% phosphoric acid).

Atrazine, sulfentrazone, and the surrogate propanil were quantified using an Agilent 1100 Series HPLC with a C18 scalar column (4.6 mm × 250 mm, 5 μm; Agilent), a C18 guard column (4.6 mm × 12.5 mm, 5 μm; Agilent) and an ultraviolet detector set at 214 nm. Gradient elution, consisting of water acidified with phosphoric acid (0.1 %) and acetonitrile was used. Starting conditions were 50% acetonitrile, moving to 90% acetonitrile at 15 min, followed by re-equilibration to 50% acetonitrile from 21 to 30 min. The flow rate was 0.5 mL/min, and 20 μL of sample was injected. Sample chromatograms were integrated on Chemstation software and quantified using an external standard calibration curve with 7 standard values ranging from 5 μg/L to 1000 μg/L.

Bioassays

Toxicity bioassays were performed on *D. magna* (a freshwater cladoceran), *P. promelas* (fathead minnow), *L. sativa* (lettuce), *P. subcapita* (green algae), and *L. minor* (duckweed). The *P. subcapita* and *L. minor* species were purchased from Carolina Biological and were cultured at Southern Illinois University for at least 2 wk before testing began. *L. sativa* seeds were purchased from Livingston Seed Company. Both *D. magna* and *P. promelas*

were from existing cultures at Southern Illinois University that were originally obtained from the USEPA.

All test species were exposed to the herbicides individually and in mixtures. All media was spiked in bulk with the compound(s) of interest using an acetone carrier and homogenized for at least 5 min before being distributed among replicates. Dosing concentrations were derived from preliminary experiments or the literature. For compounds and species for which no toxicity was expected, environmentally relevant worst-case scenario maximums were used based on literature data. All tests included solvent controls with the largest volume acetone used in the specific exposure (<0.1%) and negative controls (test water only). Each concentration had 3 to 5 replicates. For equipotent mixture bioassays, analyte concentrations were determined by adding one-half of a toxic unit (TU; concentration expressed relative to toxic criterion concentration; e.g., 1 $TU = EC50_{concentration}$) from each individual compound to determine the expected mixture toxicity (Equation 1), where Atz and Sulf denote atrazine and sulfentrazone, respectively [5]

$$1TU = \frac{1}{2}TU_{Atz} + \frac{1}{2}TU_{Sulf} \quad (1)$$

Water chemistry parameters including pH and dissolved oxygen, were measured daily in control beakers during the duration of the bioassays, using a Yellow Springs Instruments 63 meter. Acceptable pH values ranged from 6 to 8 unless indicated below, and acceptable dissolved oxygen values were above 5 mg/L [17]. Herbicide concentrations were determined by analyzing composites of the test water at the beginning and the end of the bioassay, with the average of the 2 time points being reported.

For *D. magna* acute toxicity bioassays, procedures were adapted from the USEPA methods [18] using mortality as the endpoint. Ten neonates (< 24 h old) per replicate were distributed into 3 replicate 600-mL beakers filled with 250 mL of spiked moderately hard water [19] at a single atrazine (971 μg/L or 1140 μg/L) or sulfentrazone (1330 μg/L or 1490 μg/L) concentration for individual and mixture tests, respectively. The *D. magna* were exposed to the herbicides for 48 h using a 16:8-h light:dark photocycle in a Precision 818 incubator (Thermo Scientific) at 20 °C (± 1 °C). The neonates were not fed during the bioassays.

The *P. promelas* acute toxicity procedures were adapted from the USEPA [20] using mortality as the endpoint. Larval fathead minnows (< 24 h old) were used for testing, and 10 larvae were distributed into each 600 mL beaker filled with 250 mL of spiked moderately hard water. Three replicates of 3 concentrations of atrazine (245–1090 μg/L) or sulfentrazone (230–968 μg/L) were used. For the mixture test, fathead minnows were exposed to 1 mixture concentration of 1290 μg/L atrazine and 1100 μg/L sulfentrazone. The exposure period was 96 h using a 16:8-h light:dark photocycle in an incubator at 23 °C (± 1 °C). At the end of the bioassay, the endpoint was assessed, and fish were removed from the test chambers, euthanized, and disposed of following an approved Institutional Animal Care and Use Committee method.

The *L. sativa* seeds were tested using procedures modified from Wang [21] with germination as the endpoint. Ten *L. sativa* seeds were placed in clean 10 cm disposable Petri dishes containing Whatman No. 1 filter paper with 5 mL of test solution. Five replicates of 5 concentrations of either atrazine (16.0–212 μg/L) or sulfentrazone (16.3–211 μg/L) were used. One mixture concentration was used: 1090 μg/L of atrazine and 968 μg/L of sulfentrazone. Each container was incubated at 25° C (± 1 °C) in the dark for 120 h. Bioassays were conducted in the dark to simulate natural germination that occurs underground.

The *P. subcapita* bioassays were conducted using static procedures adapted from the USEPA [22]. Chlorophyll *a* was the endpoint for this species. A cell density of 10 000 cells/mL was added to each 20-mL serum bottle of spiked freshwater algal nutrient media (initial pH, 6.8 ± 0.1). Cell counts were verified using a Palmer cell and microscope. Four replicates of 7 concentrations were used for the individual bioassays (Supplemental Data, Table S1) and 4 replicates of 6 concentrations were used in the mixture bioassays (Supplemental Data, Table S2). Tests were conducted for 96 h under a continuous light intensity of 4300 lux at 24 ± 1 °C. To measure chlorophyll *a* concentrations, a 5-mL sample containing green algae was analyzed using a fluorescence spectrophotometer. The 5-mL sample was centrifuged to condense the algal cells and re-dispersed in methanol. This algae and methanol sample was heated to 65 °C for 5 min to extract the chlorophyll *a*. After a 24-h dark period, the sample was centrifuged to isolate cell debris from the solution. An aliquot of the supernatant was analyzed by measuring fluoresce with an excitation wavelength of 436 nm and an emission wavelength of 680 nm [23].

The *L. minor* bioassays were conducted using static-renewal procedures adapted from the USEPA [24]. The endpoint used for the *L. minor* bioassays was percent inhibition of the specific growth in terms of frond number and surface area of the fronds [24]. Bioassays were run in glass jars, with 4 plants (5 d old and 14 to 16 fronds total) per jar and 55 mL of spiked Swedish standard *Lemna* growth media (initial pH, 6.5 ± 0.1 [25]). Four replicates of 7 concentrations per compound or mixture were used (Supplemental Data, Tables S1 and S2). Bioassays were conducted in an incubator under continuous cool-white fluorescent lighting at 25.0 °C (± 1.0 °C) for 168 h, to maximize growth potential. The surface area of fronds was measured using images collected by an Olympus Stylus Tough 6020 digital camera and using color threshold and area analysis by ImageJ software (Ver 1.48; National Institutes of Health).

Calculations

The time required for 50% (DT50) and 90% (DT90) of the initial herbicide concentrations to dissipate from the soil were calculated for each growing season using an exponential regression of the mean soil concentrations versus time in days (Equation 2 [26])

$$C = A_1 exp^{(xt_1)} \quad (2)$$

where C represented the mean soil concentrations at a time x (in days after application), and the initial herbicide concentration (A_1) and the dissipation rate constant (t_1) were calculated from the regression for each compound. Dissipation was measured using the application date as time 0 through 180 d after application.

Two mathematical models exist to predict mixture interactions: the concentration addition (CA), and independent action models (IA) [5]. The CA model assumes that all toxicants contribute equally to joint toxicity, because they have the same

or related modes of action. The toxicity of the mixture was predicted using Equation 3 [5]

$$\mathrm{EC}x_{mix} = \left(\sum_{i=1}^{n} \frac{p_i}{\mathrm{EC}x_i} \right)^{-1} \quad (3)$$

where $\mathrm{EC}x_{mix}$ was the total concentration of the mixture that causes x effect, p_i was the proportion of component i in the mixture, and $\mathrm{EC}x_i$ was the concentration of component i that would cause x effect.

The IA model assumes no interaction between toxicants, because they have different modes of action, which may occur if the toxicants target different receptor sites and act independently of one another. The effect of the mixture was predicted using Equation 4 [5]

$$E(c_{mix}) = 1 - \prod_{i=1}^{n} (1 - E(c_i)) \quad (4)$$

where $E(c_{mix})$ was the predicted effect of the mixture, and $E(c_i)$ was the effect expected from component i.

Both CA and IA models assume the use of equipotent mixtures and are best used to analyze quantal data. A model deviation ratio was used to determine which model (CA or IA) best fit the mixture toxicity data. A model deviation ratio was calculated using the model predicted EC50 value divided by the observed EC50 [12].

A risk quotient (RQ) was calculated to evaluate the potential effects of each herbicide on the chosen species. Risk quotients were calculated by dividing the environmental concentration by the benchmark toxicity concentrations determined in the laboratory (e.g., LC50 or EC50 values determined from the individual bioassays) for the test species (Equation 5 [3]).

$$\mathrm{RQ} = \frac{[\text{environment}]}{[\text{benchmark}]} \quad (5)$$

With risk quotient values $<<1$, it was assumed that there was little to no risk for the species at the concentrations found in the environment. A risk quotient value near 1 means there was potential for risk, and any value $>>1$ showed high-risk for that species.

A second approach to estimating risk was used when evaluating chronic or sublethal endpoints (e.g., growth). This approach compared stressor–response profiles with a range of observed exposure concentrations for each herbicide to determine a margin of safety and the 10th percentile (MOS10) [3]. The MOS10 calculates the degree of overlap between the distribution of exposure concentrations and the stressor–response profile by dividing the sensitivity concentration at which 10% of the population responds (EC10) by the environmental exposure concentration at which 90% of the measured concentrations were lower (EEC90) [3]. A MOS10 value below 1 indicates risk, and a value at or above 1 indicates little to no risk to nontarget species. The EC10 values were calculated using Probit analysis. The EEC90 values were calculated by plotting the distribution of measured herbicide concentrations from the field study and obtaining an equation for the linear regression.

Statistical analysis

All statistical analyses for laboratory bioassays were conducted using Toxcalc (Tidepool Scientific Software). Solvent control data were compared with negative control data using Dunnett's test to confirm no statistical difference between controls. Growth estimates were also compared with controls to evaluate percent inhibition. All ECx values were statistically derived using Probit analysis. The EC50 values of the individual compounds in the mixture were determined by multiplying the mixture EC50 value by the proportion of the individual compound in the mixture.

Quality assurance and quality control procedures

Quality control samples were extracted and analyzed along with each batch of field samples (18 samples per soil batch). Acceptable recoveries for quality controls were from 80% to 120%. Quality control samples included an extraction blank, a laboratory control spike, a matrix spike, a matrix spike duplicate, and a matrix blank. Method detection limits for field samples were determined by extracting a spiked reference soil and 2 different water matrices, using 7 replicates each, from sites in Carbondale (IL, USA) absent of atrazine and sulfentrazone. The method detection limit samples were spiked with 50 ng of each herbicide and values were determined by multiplying the standard deviation of samples by the Student's t-value that corresponds to the number of replicates (e.g., $t = 3.14$ for replicate value of 7). To set reporting limits, the method detection limits for each compound were multiplied by 3. In soil, reporting limits were set at 21.4 ng/g dry weight for atrazine and 25.7 ng/g dry weight for sulfentrazone. For water matrices, method detection limit were set at 0.24 μg/L for atrazine and 0.09 μg/L for sulfentrazone.

RESULTS

Field study

Both herbicide concentrations decreased throughout the year in all matrices after the last application (Figures 1 and 2). The highest mean soil concentration of atrazine was 144 ng/g dry weight (ranging from 45.8 ng/g to 714 ng/g dry wt) in 2013, and sulfentrazone was 318 ng/g dry weight (93.9–722 ng/g dry wt) in 2014 (Figure 1). The DT50 of atrazine in soil in 2013 was 67 d, and the DT90 was 96 d. However, due to the double application, the exponential regression did not fit well ($r^2 = 0.48$). A better estimate of the dissipation time for atrazine in soil, based on the second application only, was DT50 = 22 d

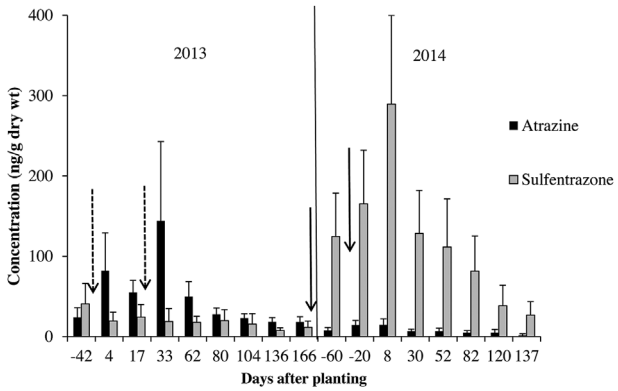

Figure 1. Atrazine and sulfentrazone surface soil concentrations for the 2013 and 2014 field seasons (ng/g dry wt; $n = 18$; +1 standard deviation). The field was in a corn/soybean yearly rotation, with 2013 starting as corn. Negative values on the x-axis indicate days prior to planting in each year. Dashed arrows represent atrazine applications, and solid arrows represent sulfentrazone applications.

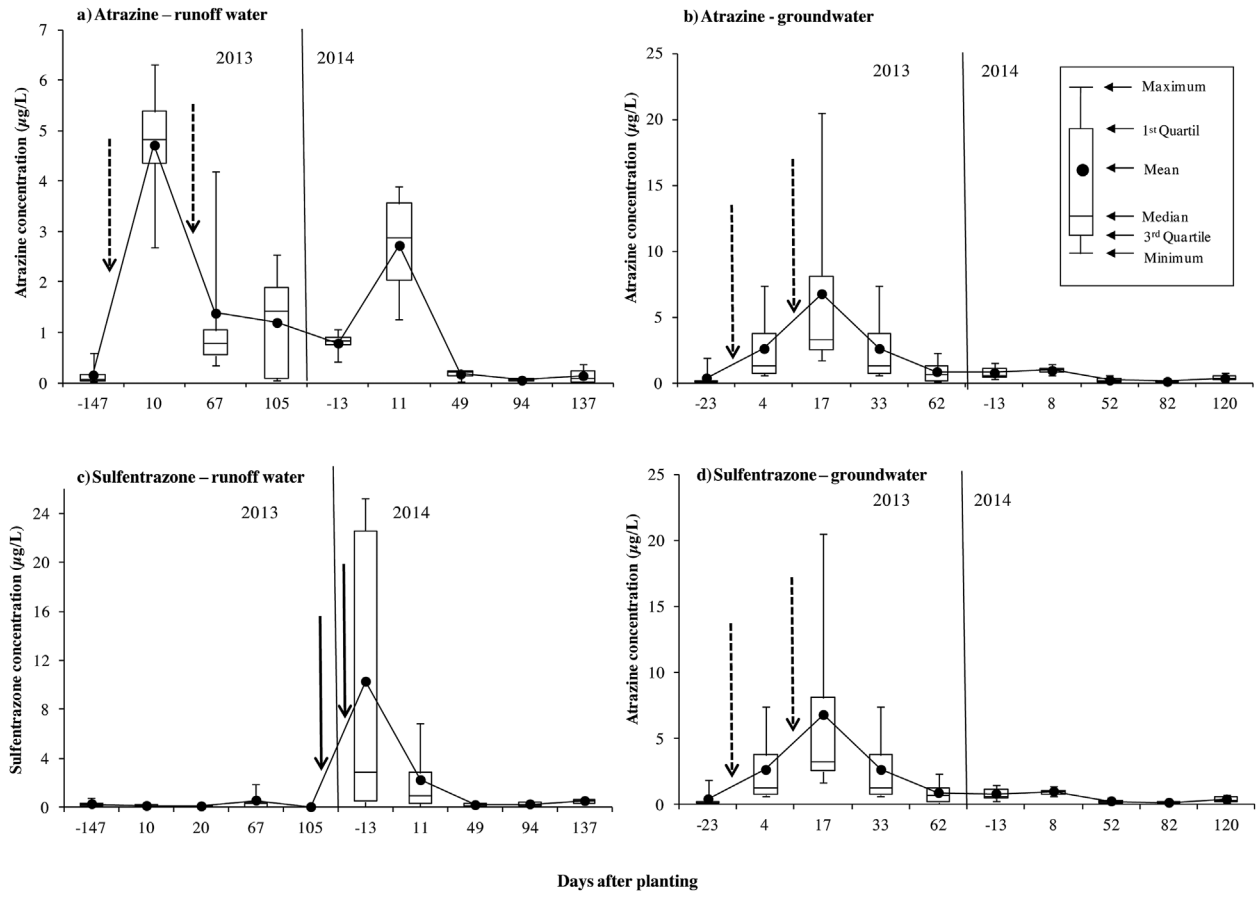

Figure 2. Atrazine concentrations in (**a**) runoff water ($n = 5$) and (**b**) groundwater ($n = 6$), and sulfentrazone concentrations in (**c**) runoff water ($n = 5$) and (**d**) groundwater ($n = 6$) for the 2013 and 2014 growing seasons. Negative values on the *x*-axis indicate days prior to planting in each year. Dashed arrows represent atrazine applications, and solid arrows represent sulfentrazone applications.

and DT90 = 74 d ($r^2 = 0.95$). The DT50 of sulfentrazone in soil in 2014 was 43 d, and the DT90 was 141 d ($r^2 = 0.94$). In both cases, atrazine and sulfentrazone concentrations were high enough after application that, although they experienced several dissipation half-lives, they were detected after harvest. High sulfentrazone concentrations in soil were found in field samples throughout 2013 from an application in April 2012. This led to a co-occurrence of the 2 herbicides in soil throughout 2013 and 2014, with peak concentrations occurring in spring 2013 and 2014 (Figure 1). This peak in co-occurrence concentrations was also observed in both water matrices in spring 2013 and 2014 (Figure 2).

The highest runoff water concentration for atrazine was 1620 µg/L after a direct application to the runoff water sampler in June 2013. The highest mean atrazine concentration in runoff water, excluding the direct spray event, was 4.72 µg/L (ranging from 2.70 µg/L to 6.32 µg/L; Figure 2). The highest mean atrazine concentration in groundwater was after the 2013 application with a concentration of 6.77 µg/L (ranging from 2.51 µg/L to 20.5 µg/L; Figure 2). Sulfentrazone runoff water concentrations peaked at 25.3 µg/L, with a mean concentration of 10.3 µg/L (0.056–25.3 µg/L) directly after spring application in 2014 (Figure 2). The highest mean sulfentrazone groundwater concentration was 7.5 µg/L (2.5–21.6 µg/L; Figure 2).

Bioassays

In *D. magna* and *P. promelas* bioassays, all control mortality was below 20%. The growth factor in *P. subcapita* assay was 134, which was measured as the ratio of the average chlorophyll *a* concentration in the controls at the end of the assay to that at the beginning of the assay. This value was verified by Palmer cell counts. The doubling time in the controls for the *L. minor* bioassay was 2.87 d^{-1} by frond count and 2.98 d^{-1} by surface area. No acute toxicity was observed for either compound, nor for the binary mixture to *D. magna* or *P. promelas* at concentrations up to 1490 µg/L. For *L. sativa*, atrazine, sulfentrazone, and the binary mixture had no effect on germination at concentrations up to 1090 µg/L. In terms of inhibition of *P. subcapita* growth, atrazine and sulfentrazone had similar toxicity (Supplemental Data, Table S3). The EC50 in the binary mixture was 199 µg/L (95% confidence interval, 142–275 µg/L; Supplemental Data, Table S3). The experimental model of the *P. subcapita* mixture bioassay was compared with CA and IA model values in Figure 3a. The model deviation ratio value for the CA model was 0.65, and the model deviation ratio value for the IA model was 0.94 (Supplemental Data, Table S4). Therefore, the *P. subcapita* data best fit the IA model, suggesting that atrazine and sulfentrazone effects are independent of one another. Individually, atrazine was less toxic to *L. minor* than sulfentrazone by frond count and surface area endpoints. The surface area endpoint indicated somewhat higher sensitivity than the frond count (Supplemental Data, Table S3). Figure 3b and c depicts the experimental model of the *L. minor* mixture bioassay and compares it with CA (Equation 2) and IA (Equation 3) model values using percent inhibition of specific growth as the effect. The model deviation ratio values were 0.69 and 0.78 (by frond count) for the CA and IA models and 0.75 and 0.88 (by surface area) for the CA and IA models,

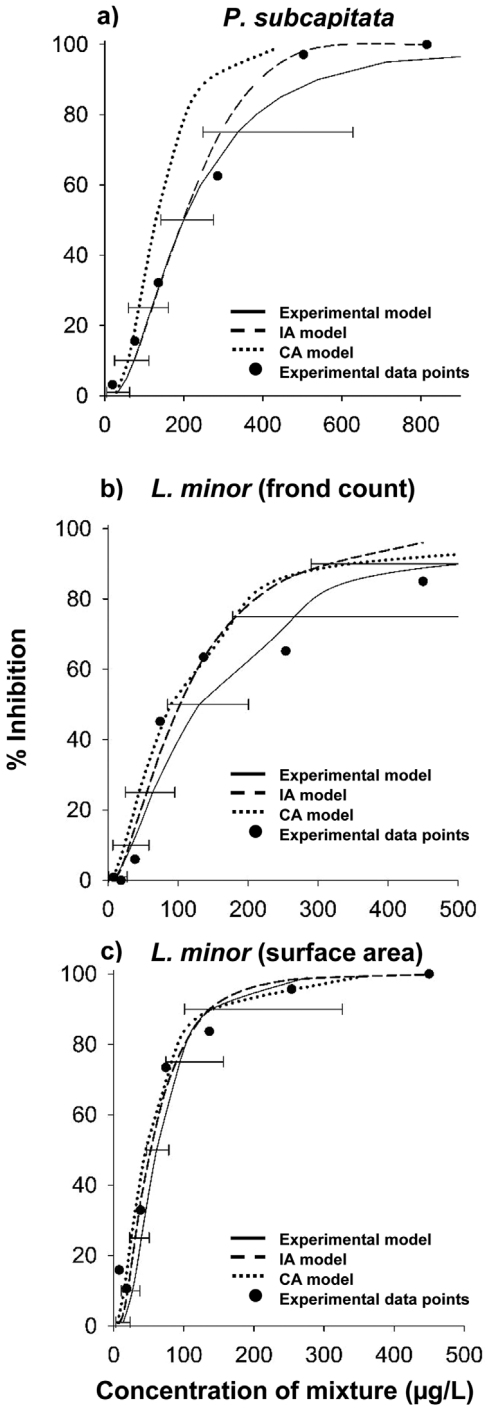

Figure 3. Experimental and model-derived dose–response curves of mixtures of atrazine and sulfentrazone to (**a**) *Pseudokirchneriella subcapita,* (**b**) *Lemna minor* with a frond count endpoint, and (**c**) *L. minor* with a surface area endpoint. Experimental data points (solid circles) are plotted for 1%, 10%, 25%, median, 75%, 90%, and 99% effect concentrations, and the solid line was calculated by the maximum likelihood Probit analysis. Horizontal error bars represent 95% confidence intervals on the modeled experimental data. IA = independent action; CA = concentration addition.

respectively, indicating the *L. minor* data best fit the IA model (Supplemental Data, Table S4).

Risk was calculated using both risk quotients and margin of safety values. For the 2 species in which toxicity was observed in the laboratory (*P. subcapita* and *L. minor*), the risk quotients were below 1 regardless of the endpoint used. During the direct spray event of atrazine, however, the risk quotient values far exceeded 1 for both species (Table 1). Additionally, the MOS10 values for these species were above 1, further indicating minimal risk. It should be noted, however, that the MOS10 for *L. minor* exposed to sulfentrazone was 1.1, which is close to the 1.0 benchmark. Therefore, there is some potential risk to *L. minor* when exposed to sulfentrazone.

DISCUSSION

Field study

Although dissipation rate and environmental half-life are used synonymously for the purposes of this manuscript, they differ slightly. Dissipation time refers to the time it takes for a pesticide to be transformed on-site (e.g., microbial degradation) or transported off-site (e.g., leaching, and runoff), assuming no specific degradation model; environmental half-life, however, assumes these processes occur under first-order degradation kinetics. The USEPA [4] reported a range of environmental half-life values for atrazine between 13 d and 261 d, whereas other studies showed a range of environmental half-life values from 41 d to 385 d [26,27], and the present study estimated a DT50 of 22 d. The sulfentrazone DT50 in the present study was 43 d, which was relatively short compared with the environmental half-life of 113 d reported by Ohmes et al. [28]. The median atrazine DT50 values in soil found in the present study were more than twice as long as the longest surface water values found in the literature (20 d [26]); sulfentrazone comparisons were even more drastic, with surface water DT50 values of 1 h compared with the shortest soil DT50 value of 14 d [29]. It is apparent that degradation of atrazine and sulfentrazone occurs more rapidly in water matrices than in soil, and transport of both compounds from soil to water matrices occurs; therefore, herbicides detected in water matrices were likely to be freshly transported from the soil.

Relatively long soil DT50 and DT90 values are possible for both atrazine and sulfentrazone in soil, which increases the likelihood for co-occurrence of the 2 herbicides. This can then lead to leaching and runoff of the herbicides, causing co-occurrence in water matrices. Co-occurrence was observed in both soil and water matrices in the present field study. The co-occurrence of atrazine and sulfentrazone occurred at every soil sampling event, with the highest co-occurrence concentrations occurring when atrazine was applied for the first time, with average soil concentrations of 82.0 ng/g dry weight and the average sulfentrazone soil concentration was 19.3 ng/g dry weight. In runoff water, co-occurrence concentrations peaked after atrazine application as well (excluding the direct spray event), when the mean atrazine concentration was 4.72 µg/L and the mean sulfentrazone concentration was 0.15 µg/L. The mean atrazine groundwater concentration was 2.73 µg/L after sulfentrazone peaked after application at 27.4 µg/L; however, atrazine concentrations were likely increased as a result of shared groundwater reservoirs from adjacent agricultural fields, planted in corn. This co-occurrence of both herbicides in soil and water matrices justifies the need for mixture bioassays. It is likely that nontarget species will encounter both compounds, either in soil or in ponds and streams fed by groundwater and runoff water; however, exact overlapping concentrations were not represented in the standard equipotent mixture bioassays used in the present study.

Bioassays

No acute toxicity was observed for either *D. magna* or *P. promelas* for either compound or the binary mixture at

Table 1. Risk quotient (RQ) values for *Daphnia magna, Pimephales promelas*, *Lactuca sativa*, *Pseudokirchneriella subcapita*, and *Lemna minor* using both the direct atrazine spray event concentration and the highest mean concentrations for atrazine and sulfentrazone in runoff water

Species	Atrazine RQ	Sulfentrazone RQ	Direct spray atrazine RQ
D. magna	Not toxic[a]	Not toxic	Not toxic
P. promelas	Not toxic	Not toxic	Not toxic
L. sativa	Not toxic	Not toxic	Not toxic
P. subcapita	0.032	0.115	11.0
L. minor[b]	0.034	0.395	13.5

[a]"Not toxic" denotes lack of toxicity of the compound; RQ < 1 denotes little to no risk; RQ > 1 denotes high potential for risk.
[b]The more conservative median effect concentrations measured using the surface area endpoint were applied.

concentrations up to 1490 μg/L. Although atrazine is not thought to be acutely toxic to animals, atrazine can affect plants and animals via narcosis at high concentrations [30]; therefore, it was important to evaluate whether these compounds individually or as mixtures had the potential to cause lethality to nontarget invertebrates or fish at environmentally relevant concentrations. It should be noted, however, that although sublethal effects for these species were outside of the scope of this research, it is possible that these chemicals individually or in mixtures could illicit sublethal effects on these animals. The main modes of action of atrazine and sulfentrazone in plants are the inhibition of photosynthesis through the inhibition of photosystem II [5] and protoporphyrinogen oxidase [9], respectively. Therefore, it was predicted that these 2 herbicides would have adverse effects on nontarget plant species; however, there were no observed effects on the germination of *L. sativa* for either compound or the binary mixture. This was likely attributable to the fact that during germination, the *L. sativa* seeds are using nutrient seed reserves for energy and not utilizing energy from photosynthesis [31]. The fact that no effects were observed for *L. sativa* germination is important, because germination is correlated with herbicide application in the planting of field crops, and pre-emergent crops could be damaged if atrazine or sulfentrazone had effects on seed germination.

In contrast, atrazine and sulfentrazone did have adverse effects on *P. subcapita* growth. Atrazine had an EC50 value of 147 μg/L, comparable to median lethal atrazine concentrations (LC50) and median atrazine effect concentrations (EC50) for *P. subcapita* in the literature, which ranged from 26 [7] to 235 μg/L [6]; however, a number of toxicity endpoints were examined in these studies (i.e., algal photosynthetic effects, algal lethality, and algal growth inhibition). Sulfentrazone had an EC50 value of 89.6 μg/L. The sulfentrazone EC50 value was more than 3 times higher than the maximum concentrations found in water matrices of the present field study. The atrazine EC50 value was more than 30 times higher than the maximum water concentration (excluding the direct spray event); therefore, neither EC50 value was environmentally relevant.

In addition to adverse effects on *P. subcapita*, the herbicides also had adverse effects on *L. minor* frond counts, with EC50 values of 139 μg/L and 58.8 μg/L for atrazine and sulfentrazone, respectively. Fairchild et al. [6] conducted toxicity bioassays on *L. minor* exposed to atrazine and found an EC50 value of 153 μg/L; however, these bioassays [6] used a shorter testing period and noncontinuous photocycle. Overall, *L. minor* was the more sensitive of the 2 species of nontarget aquatic plants to both herbicides. It is hypothesized that *L. minor* was more sensitive, because it is vascular, actively incorporating contaminated water into its system, compared with *P. subcapita*, which needs to absorb the herbicides intracellularly for them to cause an effect [9]. However, neither atrazine nor sulfentrazone EC50 values for *L. minor* were near environmentally relevant concentrations; the EC50 values were at least twice as high as the water concentrations found in the field of the present study. In addition, it should be noted that the pH of the media in the bioassays (6.5 to 6.8) was near the acid dissociation constant of sulfentrazone (6.56) [10], and extrapolating toxicity effects to the nontarget organisms is likely to be contingent on local pH in the aquatic environment.

The effects of the mixture of atrazine and sulfentrazone on *P. subcapita* and *L. minor* were assessed, because the herbicides were observed to co-occur in environmental samples. Both herbicides inhibit photosynthesis; however, they do not compete for the same target sites [5,9]. The IA of the herbicides was confirmed when reviewing the CA/IA models for *P. subcapita* (Figure 3a). The *P. subcapita* model deviation ratio value for the IA model was 0.94. Similarly, the *L. minor* model deviation ratio value using the surface area endpoint for the IA model was 0.88 (Figure 3; Supplemental Data, Table S4). Although the mixture bioassays in the present work were better modeled by IA, most regulatory agencies recommend the use of CA because it tends to provide a more conservative estimate of risk [32]. This difference attests to why it is important to conduct mixture bioassays and not use only modeled data.

Risk estimation

The risk associated with the application of atrazine and sulfentrazone was evaluated in 2 ways. First, risk quotients were calculated using the highest mean runoff water concentration for the respective compound and the EC50 values for the aquatic plant species (Table 1). With risk quotient values $<<1$, it was assumed that there was little to no risk for the species at the concentrations found in the environment. A risk quotient value close to 1 means there was potential for risk, and any value $>>1$ showed high-risk for that species. The risk quotient values for atrazine were evaluated using both the direct spray event concentration and the highest mean runoff water concentration

Table 2. Margin of safety (MOS10) values for *Pseudokirchneriella subcapita* and *Lemna minor* in runoff water

Species	Herbicide	EC10 (μg/L)	EEC90 (μg/L)	MOS10[a]
P. subcapita	Atrazine	57.25	4.99	11.5
L. minor	Atrazine	24.36	4.99	4.9
P. subcapita	Sulfentrazone	3.40	8.29	7.8
L. minor	Sulfentrazone	9.19	8.29	1.1

[a]MOS10 > 1 means a chemical is relatively safe; MOS10 < 1 indicates risk. EC10 = concentration that causes an effect on 10% of the population; EEC90 = environmental exposure concentration at the 90th percentile, not including the direct atrazine spray event.

other than what was seen in the direct spray. The atrazine risk quotient values for the nondirect spray event were 0.032 for *P. subcapita* and 0.034 for *L. minor*, suggesting no risk at the 50% effect level. Direct spray event risk quotient values for atrazine were 11.0 and 13.5 for *P. subcapita* and *L. minor*, respectively (Table 1). These values are far above 1, suggesting high risk, but should be considered an absolute worst-case scenario. Sulfentrazone risk quotient values were 0.115 for *P. subcapita* and 0.395 for *L. minor* (Table 1). Overall, excluding the direct spray event, there appears to be little to no risk associated with atrazine and sulfentrazone exposure at the 50% effect level.

To evaluate a more conservative estimate of risk, a second approach was used to characterize risk using the 10% effect level [3]. The MOS10 is a risk estimation technique that allows the probability of risk to be determined throughout the entire year. The MOS10 was calculated by taking the effective concentration at the 10th percentile divided by the 90th percentile of cumulative exposure distribution, which was the categorized frequency of environmental concentrations throughout the growing season [3]. This approach was advantageous, because it allowed risk to be characterized not only by the highest exposure concentration, as with risk quotients, but by the concentrations that have the potential to cause sub-lethal effects to nontarget species. A MOS10 >1 indicates a chemical is relatively safe, whereas a MOS10 <1 indicates risk. Atrazine MOS10 values, calculated with environmental concentrations (not including the direct spray event) were 11.5 and 4.9 for *P. subcapita* and *L. minor*, respectively (Table 2 and Figure 4). These values were greater than 1, meaning atrazine at environmentally measured concentrations posed little risk to the growth of either aquatic plant species at the 10% effect level. The MOS10 value for sulfentrazone effects on *P. subcapita* was 7.8, and the value for effects on *L. minor* was 1.1 (Table 2).

Although MOS10 values could not be calculated for the mixture, the effects of atrazine and sulfentrazone via IA that were observed in both aquatic plant species would suggest that the 2 compounds pose little to no increased in risk in combination. The smaller MOS10 values for sulfentrazone suggested that it posed the greater risk of the 2 herbicides, and

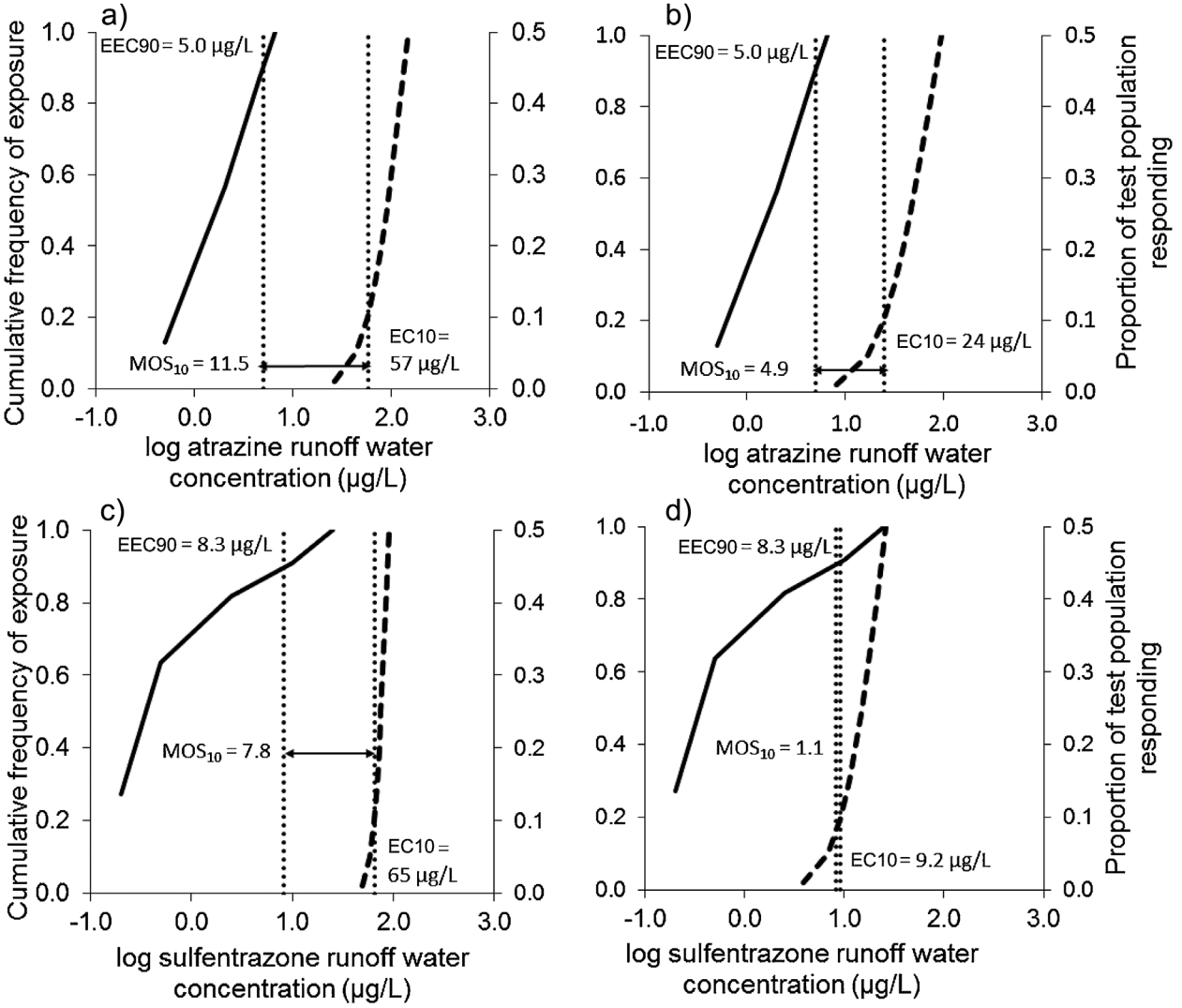

Figure 4. Risk probability curves (solid line) for atrazine ($y = 0.78x - 1.15$; correlation coefficient, $R^2 = 0.99$) and sulfentrazone ($y = 0.31x - 0.31$, $R^2 = 0.88$) in runoff water. A MOS10 <1 indicated risk, and a MOS10 >1 indicated a chemical was relatively safe. Effects are shown by dashed line (- −) for *Pseudokirchneriella subcapita* (**a**,**c**) and *Lemna minor* (**b**,**d**) using the surface area endpoint. EC10 = concentration at which 10% of the population is effected; EEC90 = environmental exposure concentration at the 90th percentile; MOS10 = 10th percentile of margin of safety, calculated by dividing the EC10 by the EEC90.

the binary mixture of atrazine and sulfentrazone posed the highest risk to aquatic plant growth.

Some uncertainties were encountered in the present study. First, the risk evaluation techniques used (e.g., risk quotient and MOS10) were based on a chosen toxicological endpoint. The risk quotient estimate is based on a lethal endpoint, whereas MOS10 is based on a sublethal endpoint. These estimates were biased based on the chosen endpoint and different endpoints could produce more or less conservative estimates. Second, the binary mixture of atrazine and sulfentrazone was an equipotent mixture, which was not equivalent to the co-occurrence concentrations found in the present field study. Exact overlapping concentrations were not accurately represented in mixture bioassays; therefore, it is only assumed that the 2 herbicides will interact with one another in the same manner in the field setting. It would be beneficial to conduct mixture bioassays using the environmentally relevant co-occurring concentrations, to confirm the synergistic potential of the herbicides in the environment. Finally, the lab bioassays were conducted with neat materials, whereas applications in the field were completed with a formulation including inert ingredients. To address this issue, the parent compound was quantified in both lab and field studies.

CONCLUSIONS

Environmental concentrations in soils were high enough to allow residues of both compounds to still be detectable nearly 1 yr after their respective application. Compounds also co-occurred during the spring of 2013 and 2014 in the water matrices, justifying the need for mixture bioassays. Although sub-lethal effects at the 50% effect level of growth were observed in nontarget plant species *P. subcapita* and *L. minor* for both herbicides, these values were above environmental concentrations detected in the present field study. Furthermore, the risk quotient values suggest that there is little to no risk associated with atrazine and sulfentrazone exposure concentrations at the 50% effect level, except in the rare case of a direct spray event of atrazine. Using the more conservative MOS10 risk characterization approach, it was determined that there is little risk to nontarget plant species, with MOS10 values >1. Although the co-occurrence of atrazine and sulfentrazone was observed, and synergistic effects were observed in the equipotent binary mixture bioassays for *P. subcapita* and *L. minor*, it is believed that there is minimal increase in risk potential due to the co-occurrence.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3664.

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