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Are safeners safe? An assessment of the toxicity of benoxacor, mono-chlorinated benoxacor, S-metolachlor, and a mixture to *Chironomus riparius* within benthic microcosms

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ABSTRACT: The environmental effects of safeners, chemicals that protect crops from herbicide toxicity, are largely unknown. Safeners are considered inert ingredients added to many classes of herbicides. We compared the toxicity of the dichloroacetamide safener benoxacor, with its degradation product (mono-chlorinated benoxacor), a typically paired herbicide (S-metolachlor), and a mixture of S-metolachlor and benoxacor, to larvae of *Chironomus riparius* in benthic microcosms containing natural, iron-rich sediment. Larval *C. riparius* were exposed to these four chemicals in spiked sediments during chronic 28-day experiments. High concentrations (~100 mg/kg, 200 mg/kg mixture) of all four chemicals significantly affected percent adult emergence. These high concentrations of benoxacor and the S-metolachlor + benoxacor mixture reduced adult emergence rates, and high concentrations of S-metolachlor reduced male adult biomass. All four chemicals, at varying concentrations, affected hazard ratios of emerging healthily for both sexes. Benoxacor and its degradation product (mono-chlorinated benoxacor) were shown to be more toxic to *C. riparius* (based on survival, time-to-emergence, and emergence rate) at similar concentrations compared to the herbicide S-metolachlor. A transformation rate constant (k_t,) of 0.06 ± 0.03 d^{-1} was determined for benoxacor within a spiked-sediment microcosm and the half-life for benoxacor was calculated as 11.6 ± 3.9 d. The toxicity experiments and evaluation of benoxacor persistence under microcosm conditions provides useful insight into the ecotoxicological effects of dichloroacetamide safeners.

Key words: safener, *Chironomus*, dichloroacetamide, benoxacor, S-metolachlor

INTRODUCTION

Herbicides are capable of damaging crop plants while concurrently retarding or preventing weed growth [1]. "Safeners," chemicals that protect crops from herbicidal injury when mixed into formulation with active herbicides, have become more prevalent over the past few decades [1,2]. The dichloroacetamide class of safeners, which includes benoxacor, dichlormid, furilazole, and AD67, is used to protect corn from the thiocarbamate and chloroacetamide classes of herbicides (Table 1) [2,3]. The dichloracetamide safeners and chloroacetamide herbicides are structurally similar, differing primarily in the number of chlorines attached to each molecule.

Dichloroacetamide field application rates are estimated at over 2 million kg/yr in the United States [4], exceeding application totals of many active herbicides [5]. Nevertheless, their environmental distribution, fate and toxicity have not been well studied [4,5]. Considered "inert" ingredients in herbicide formulations [2], safeners are registered differently than herbicide active ingredients in the United States [2]. Although fate and effects data are required for inert ingredients during the registration process, there is a large knowledge gap regarding ecological effects studies [5], including chronic aquatic sediment toxicity studies, for the dichloroacetamide safeners. Given the extensive use of safeners in the U.S. and elsewhere, this lack of ecotoxicity data embodies a significant uncertainty in assessing the ecological risks of these agrochemicals [4]. In anaerobic reaction chambers in the presence of ferrous iron and iron oxide minerals, dichloracetamide

92 safeners can undergo reductive dechlorination, transforming into products that
93 more closely represent their active herbicidal counterparts (Figure 1) [5].
94 Currently, only the active ingredient of an herbicide is required to undergo
95 chronic, aquatic sediment ecotoxicological studies for registration in the U.S., but
96 only under certain conditions [6]. Chronic whole sediment studies are required for
97 the technical grade of the active ingredient (TGAI) only if the degradation half-life of
98 the pesticide is less than 10 days in aerobic soil [6] and if one of a few other
99 conditions applies, one being that the octanol-water partition coefficient (log K_{ow}) is
100 ≥ 3 [6]. For the dichloroacetamide safener benoxacor, log K_{ow} is estimated to be over
101 3 [5] and estimated half-lives range from 5 to 49 days in aerobic soil [2,7].
102 "Acute" 48- to 96-hour, and "chronic" multiple weeks-long toxicity tests have
103 demonstrated the effects of the common herbicide S-metolachlor on many aquatic
104 organisms [8,9]. Yet, the toxicity of this product's safener, benoxacor, remains
105 somewhat undetermined [8,9]. Liu et al. (2006) determined a lowest-observable-
106 effect-concentration (LOEC), the lowest concentration producing effects that were
107 significantly different from control responses, on organism growth of juvenile
108 *Daphnia magna* to S-metolachlor to be 1 mg/L in a 21-day fresh water assay [10].
109 United Stated Environmental Protection Agency (USEPA) (2013) found a LOEC in
110 organism mortality to S-metolachlor to be 20.3 mg/L in a 21-day freshwater study
111 of *Daphnia magna* [11]. Investigations into the toxicity of benoxacor to terrestrial
112 organisms *Folsomia candida* and *Poecilus cupreus* determined that for these
113 organisms benoxacor presented low environmental risk [12]. However, the effects
114 of benoxacor on aquatic organisms are likely much more significant. With a lowest

115 acute LC_{50} of 0.63 mg/L for freshwater algae, and a lowest reported LC_{50} of 1.4 mg/L
116 for the freshwater fish *Ictalurus punctatus* [13], benoxacor can be classified as highly
117 [14] and moderately toxic to aquatic plants and animals, respectively [4]. Chronic
118 experiments demonstrated effects at even lower concentrations. For example,
119 benoxacor resulted in a NOEC of 0.354 mg/L in carapace length of *Daphnia magna*,
120 following a 21-day life-cycle study [13]. This is lower than what was observed
121 following S-metolachlor exposure by Liu et al. (2006) [10]. Whole sediment chronic
122 ecotoxicological tests span several weeks to detect sublethal effects of sediment-
123 adsorbing chemicals on benthic organisms [15]. An ecologically-relevant profile of
124 the safener benoxacor should include chronic sediment toxicity tests that model
125 likely environmental conditions for freshwater benthic organisms.
126 *Chironomus riparius* (Diptera: Chironomidae) larvae are common in muddy
127 environments characterized by ample organic matter, fine- to medium-grained
128 sediments and poor water quality [16,17], including streams and stagnant ditches
129 [17]. Considered an ecosystem engineer, *C. riparius* can alter the partitioning of
130 xenobiotics between sediment and the water column through sediment bioturbation
131 from larval burrowing [18]. *C. riparius* is an easily cultured, standard test organism
132 for assessing toxicity of sediment-sorbed chemicals [19]. Guidelines of the
133 Organization for Economic Cooperation and Development (OECD) provide a
134 standard bioassay for exposure of *C. riparius* to compounds incorporated into test
135 sediment [20] comprised of a mixture of quartz, kaolinite, and alpha-cellulose.
136 [20,21,22]. Because the purified kaolin clay used in standard tests is deficient in iron
137 oxides [23], surface-mediated reductive dechlorination would not occur in such a

test design. However, iron-rich substrates are common components of soils and sediments in agricultural areas, and under iron-reducing conditions characteristic of some water-logged soils and sediments, dichloroacetamide safeners could conceivably undergo reductive dechlorination.

We assessed the sensitivity of *C. riparius* to 4 chemical treatments: the safener benoxacor, its transformation product (mono-chlorinated benoxacor), S-metolachlor (the herbicide with which benoxacor is typically paired in commercial products [24]) and a mixture of S-metolachlor and benoxacor, within microcosms representative of natural, benthic freshwater habitats. The mixture concentration (~100 mg/kg each chemical) is not representative of a formulation; an initial goal was to potentially assess the mixture effects for concentration addition. A second aim of this study was to detect and model the partitioning and degradation of benoxacor in these microcosms. Emergence ratio, the number of adults emerged by the end of the study to the number of larvae originally initiated, was the measure of survival. The sub-lethal endpoints analyzed were adult emergence rate, adult body weight, and the hazard ratio of successfully emerging. The results obtained in this study help to fill a gap in the knowledge on the long-term toxic effect of these agricultural compounds on *C. riparius*, in the context of spiked-sediment.

MATERIALS AND METHODS

Test compounds

Benoxacor and S-metolachlor were obtained from Fisher Scientific, both with purities of 99.5%. The Sivey Research Group at Towson University synthesized

mono-chlorinated benoxacor for use in this project; the purity of mono-chlorinated benoxacor was 90% [25]. We exposed larvae to nominal sediment concentrations ranging from 0.01 mg/kg to 100 mg/kg dry weight (single chemicals) and from 0.02 mg/kg to 200 mg/kg dry weight (mixture) based on effects observed in preliminary range-finding studies (Supplemental Data, Table 1).

Test organisms

The culture of *C. riparius* used was established from a natural population (Mt. Washington, Baltimore City, Maryland) and maintained in the Urban Environmental Biogeochemistry Laboratory at Towson University (Towson, Maryland) since 2014. The stock culture was reared under a 16:8 h light:dark photoperiod at an ambient air temperature of 20 °C. Glass, 1-gallon rearing vessels contained shredded brown paper-towel substrate submerged in a modified Elendt M7 medium (as prescribed by OECD 218 with minor modifications) [20].

Preparation of artificial sediment

Clay and iron-rich sub-soil was field-collected (Mt. Washington, Baltimore City, Maryland) and transported to Towson University in plastic high-density polyethylene buckets. Sediment was prepared by collecting sub-soil material that passed a 2 mm sieve. With addition of sufficient deionized water, this sediment was thoroughly mixed with a Teflon-coated, drill-attached mixer. This sediment was used for all subsequent experiments. Iron content was determined by dissolving dry sediment samples into glass fusion beads, which were analyzed using a Bruker

AXS S4 Explorer wavelength-dispersive X-ray fluorescence spectrometer. Iron content was determined to be 13.4% by mass. Total carbon (TC) content was analyzed with a Shimadzu TOC-Vcsh NC Analyzer. TC of the sediment was < 0.5%. Approximately 5 kg of alpha-cellulose (Sigma-Aldrich) was added to a large quantity of sediment in order to raise the carbon content to about 30% by dry weight. Alpha-cellulose was mixed into the sediment until homogeneous using a Teflon-coated, drill-attached mixer.

Wet sediment was spiked on a dry-weight basis by allocating test substance stock solutions to a small portion of sand (5% dry weight of each treated sediment batch, Supplemental Data, Table 2). These methanolic spikes were allowed to dissipate under a fume hood before being added to each treatment's respective batch of sediment, which was then mixed and shaken by hand. Spiked sediment batches were then aliquotted to experimental replicates (1-quart glass mason jars), and the mass of spiked sediment in each replicate was recorded (Supplemental Data, Table 3).

C. riparius exposure conditions

1-quart glass mason jars were maintained at 20 °C, in a 16:8 h light:dark photoperiod (Figure 2). Approximately 2 cm of sediment was provided as substrate (weights were recorded, Supplemental Data, Table 3), and 600 mL of culture medium was slowly added as overlying water. Water and sediment were left for 1 d to allow settlement of solids before study initiation.

Rangefinders

Three range-finding studies were conducted in order to determine appropriate concentrations for the definitive exposure experiments. Rangefinder 1 included benoxacor at nominal concentrations ranging from 0.01 mg/kg to 100 mg/kg, Rangefinder 2 included nominal concentrations of benoxacor ranging from 0.01 µg/kg to 1000 µg/kg. Rangefinder 3 included S-metolachlor concentrations ranging from 0.01 µg/kg to 1000 µg/kg.

Definitive Toxicity Studies

The definitive toxicity studies were completed in duplicate experimental blocks. Both blocks contained only first-instar larvae. Block 1 of the experiment consisted of microcosms stocked with 4-three day-old larvae, 12-two day-old larvae, and 4-one day-old larvae, each. The best effort was made to use a similar age-distribution of larvae in experimental block 2. Block 2 contained 8-three day-old larvae, 8-two day-old larvae, and 4-one day-old larvae. Egg masses hatched in small beakers containing modified Elendt M7 medium and a pinch of finely ground Tetramin® flake food. First instar larvae were randomly allocated, two to three organisms at a time until replicates reached 20 individuals as described above. Following organism allocation, the light aeration supplied to each replicate was temporarily suspended for approximately 1 d and then re-administered for the duration of the study. There were two replicates of each concentration of the 4 chemical treatments, in each experimental block. Thus the first block contained 8

negative control replicates and 8 solvent control replicates; the second block contained 3 negative control replicates and 4 solvent control replicates.

During the exposure experiments, dissolved oxygen, pH, and temperature were measured approximately every 3 d until the end of the test, in rotation among all microcosms (Supplemental Data, Tables 4 and 5). Larvae were fed approximately 3 times per week with 10 mg/larvae/day crushed fish food (Tetramin®), unless accumulating food and/or fungal growth was observed on the sediment surface, at which point food was decreased or suspended for all experimental replicates at the same rate.

Observations of any dead larvae or pupae apparent on the sediment surface or in the water column were made daily, and once the first observation of an emerged adult was made, observations were made twice daily, approximately twelve hours apart for the rest of the study. Dead larvae/pupae and emerged adults were carefully removed with a pipette or forceps and stored, by replicate, in vials containing 70% v/v ethanol.

Benoxacor Spike Recovery

To quantify the partitioning of benoxacor between aqueous and sedimentary phases, the recovery of a benoxacor spike was measured over time from microcosms containing different media. Six experimental units were prepared in 250 mL glass beakers as follows: (1) 200 mL of DI water, (2) 200 mL of modified Elendt M7 medium, (3) 200 mL of DI water spiked with benoxacor, (4) 200 mL of Elendt M7 medium spiked with benoxacor, (5) spiked sand (31 g dry weight, spiked

252 with benoxacor to achieve 100 mg/kg nominal concentration), and (6) 31 g dry
253 weight of Mt. Washington sediment, spiked at the same nominal concentration.
254 These two substrate units also contained 150 mL of overlying Elendt M7 medium,
255 which was slowly added above the spiked sediment layer. Aqueous samples were
256 taken at pre-determined days for 28 days. Aqueous samples were collected using 3
257 mL plastic syringes and filtered using a 0.2 µm nylon filter. Samples were stored in 2
258 mL glass autosampler vials, in the dark at 0 °C.
259
260 *Determination of analytes in stock solutions used for dosing*
261 Quantitation of benoxacor, mono-chlorinated benoxacor, S-metolachlor, and
262 the mixture in stock solutions used for dosing the sediment in the definitive toxicity
263 experiments was performed via HPLC (Agilent 1200) with a diode array detector set
264 to 254 nm. Analyte separations (10 µL injection volume) were achieved using a
265 Poroshell 120 EC-C18 column (5 cm × 2.1 mm × 2.7 µm); an isocratic elution
266 program (50:50 vol% mixture of HPLC grade acetonitrile and 18 MΩ•cm water) was
267 employed with a flow rate of 0.55 mL/min. Retention times for mono-chlorinated
268 benoxacor, benoxacor, and S-metolachlor were 1.5 minutes, 2.0, and 2.5 minutes,
269 respectively. The mass of each stock added to sediment by dry weight and the
270 resulting nominal sediment concentrations were recorded (Supplemental Data,
271 Table 2). The same stocks were used for dosing sediment in both experimental
272 blocks based on the dry weight of the sediment batches spiked. Nominal
273 concentrations of our sediment batches were determined by the mass of those
274 stocks added to each batch of sediment, and those stocks' measured concentrations

on HPLC (Table 2). The median nominal concentrations were calculated between these two batches and are outlined in Supplemental Data, Table 2. These were near our pre-determined range of sediment concentrations, which are generally used to describe treatment levels throughout the rest of the results of this paper.

STATISTICAL ANALYSES

Endpoints analyzed

The *C. riparius* endpoints analyzed were percent adult emergence (survival), emergence rate, adult biomass, and time-to-emergence. Each endpoint was analyzed by both ANOVA and non-linear regression (three-parameter Weibull model) in order to determine experimental NOEC and LOEC values, and to attempt to clarify the dose-response model of each chemical and respective EC_X values. The assumption of normality of the data distributions was tested using the Shapiro-Wilks test, and the homoscedasticity of the data sets was tested using the Bartlett's test. When these assumptions were met, parametric tests were used, and when these tests were not met, raw endpoint data were compared to transformed endpoint data, where possible. In this situation, when treatment was a significant explanatory variable in both, the test using the raw data set was chosen. ANOVA can be robust to deviations from assumptions [26]. Alpha was always set at 0.05, and ANOVA tests were corrected for family-wise error rate by a Dunnett's test when appropriate. All statistical tests were performed in R Studio (Ver. 0.99.467). Specific analysis methods can be found in Supplemental Data.

RESULTS

Benoxacor Spike Recovery

The aqueous concentration of benoxacor recovered from microcosms either without substrate, with sand substrate, or with natural clay and iron-rich sediment substrate, was measured over 28 days (Figure 3) (Supplemental data, Table 6). When benoxacor was added to the water-only and medium-only microcosms, concentrations remained stable at approximately 80 µM (79.89±5.0 SD and 81.10±2.6 SD) measured from day 4 to day 28. When benoxacor was added as a spike to sand, aqueous concentrations gradually increased towards a plateau at 66.25 ± 4.3 SD µM (day 4 to day 28), showing some partitioning of benoxacor between the sand layer and overlying medium. In contrast, when benoxacor was added as a spike to the natural clay-rich sediment, aqueous concentrations reached approximately half as much as in the sand-spiked microcosm, (38.38 ± 4.1 SD µM) measured from day 4 to day 28, and by halfway through the experiment there was a clear decline in aqueous concentration in the natural clay sediment microcosm (Figure 3). This suggests that benoxacor was transformed (e.g., via reductive dechlorination [5]) in the microcosms containing natural sediment .

The temporal data associated with the heterogeneous systems (Figure 3, solid symbols) were fit to the kinetic models shown in Table 3 using a non-linear least squares regression analysis (Scientist 3.0, MicroMath). The results indicate that the rate constant for benoxacor desorption from sand is about 2× higher than desorption from the sand + sediment system (Table 3). Throughout the experiment, more benoxacor remained in the sediment layer than in the sand-only layer. The

transformation rate constant for benoxacor spiked into the natural sediment substrate was determined to be 0.06 ± 0.03 d^{-1} (95% CI). Given the parameters of the kinetic model for the sand + sediment + medium microcosm (Table 3), the half-life for benoxacor in sediment was determined to be 11.6 ± 3.9 d. A K_d value of ~1 for benoxacor in the sand + sediment + medium microcosm was calculated, assuming $[40\ \mu M]_s$ and a measured $[\sim 40\ \mu M]_{aq}$. With a mass fraction of about 0.3 organic content in our sediment, the K_{oc} was calculated as ~3.0.

Emergence Ratio

There was a significant effect of dose on emergence ratio for each of the four chemical exposures (Figure 4). The experimental blocks were effective in partitioning some of the random variation in all experiments except for the benoxacor exposure (Supplemental Data, Table 19). Based on the results of our ANOVA and Dunnett's test, the NOEC was at a nominal concentration of 10 mg/kg, or 20 mg/kg for the mixture, and the LOEC was at a nominal concentration of 100 mg/kg, or 200 mg/kg for the mixture. The blocking term effectively partitioned some of the random variation in emergence ratios that would otherwise have been confounded with the dose effect, if it had been possible to run all replicates simultaneously. Dunnett's tests showed significant results for the 100 mg/kg dose compared to the solvent control group for each chemical treatment: Benoxacor (P(>|t|) = 0.0017**); Monochlorinated benoxacor (P(>|t|) = <1e-04***); S-metolachlor (P(>|t|) = 0.001***); Mixture (P(>|t|) = <1e-04***).

Application of the three-parameter Weibull model further elucidated the dose-response relationship for each chemical (Supplemental Data, Table 20). However, in all four exposure scenarios, the 95% confidence interval for the EC20 extended past the 0 mg/kg point (into negative dose values) and the 95% confidence interval for the EC50 extended past our maximum tested concentrations, limiting our ability to predict these effect concentrations without extrapolating (Supplemental Data, Table 21).

Emergence Rate

In contrast to emergence ratio, the effects of mono-chlorinated benoxacor and S-metolachlor treatments on male and female emergence rates were insignificant, whereas benoxacor and the mixture did have significant negative effects on male and female emergence rates (Figure 5) (Supplemental Data, Table 22). Only the high dose group (100 mg/kg, 200 mg/kg) exposed to benoxacor and to the mixture differed significantly from the solvent control group. Dunnett's test results for these dose levels were as follows: Benoxacor males (P(>|t|) = 2.6e-03**); Benoxacor females (P(>|t|) = 3e-04***); Mixture males (P(>|t|) = <1e-04***); Mixture females (P(>|t|) = 5.7e-03**).

Non-linear regression was not useful in determining the EC10 and EC20 values, since the 95% confidence intervals were fairly or extremely large (Supplemental Data, Table 23), inhibiting our ability to predict these effect concentrations without some degree of extrapolation (Supplemental Data, Table 24).

366

367 *Adult Weight*

368 Only the males exposed to the high concentration of S-metolachlor
369 demonstrated a significant decline in adult body mass (Figure 6, Supplemental Data,
370 Table 25). There was a clear graphical decline in body mass as treatment increased.
371 The Dunnett's test for the 100 mg/kg to solvent control group comparison was
372 significant (P(>|t|) =2.19e-04***). The usefulness of non-linear regression was again
373 limited, because the EC20 and EC50 95% confidence intervals extended into
374 negative values, inhibiting our ability to predict these effect concentrations without
375 some degree of extrapolation (Supplemental Data, Table 26).

376

377 *Cox proportional hazards analyses*

378 The high treatment (100 mg/kg, 200 mg/kg mixture) of benoxacor, mono-
379 chlorinated benoxacor, S-metolachlor, and the mixture significantly decreased the
380 likelihood of successfully emerging for both males and females (Table 4) (Figure 7).
381 We were also able to detect a significant effect of the 1 mg/kg benoxacor treatment,
382 0.1 mg/kg mono-chlorinated benoxacor treatment, and 0.02 and 0.2 mg/kg mixture
383 treatments, in female likelihood of emerging successfully (Table 4).

384 Cox proportional-hazards model results produce an exponentiated
385 coefficient. [27] Since these curves are not time-to-failure (as typically modeled in
386 the literature) but time to successful, healthy emergence, the interpretation of the
387 exponentiated coefficient might seem counterintuitive. In the case of males exposed
388 to the high concentration of benoxacor, individual organisms have a 35% likelihood

of successfully emerging, on any given day, when exposed to 100 mg/kg of benoxacor, in comparison to control organisms (Table 4). There are similar hazards to females exposed to the high concentration of benoxacor (~30%), as well as to females exposed to a lower concentration of benoxacor (1 mg/kg) which have a ~57% likelihood of emerging on any given day when compared to the control organisms (Table 4). Both the males and females exposed to the high concentration of S-metolachlor face a significant decrease in the likelihood of emerging when compared to the control group (~23% and 40% when compared to the control, respectively). Males and females exposed to the high levels of mono-chlorinated benoxacor saw even smaller likelihoods of successful emergences (14% and 9%, respectively). Females exposed to the lower concentration of 0.1 mg/kg mono-chlorinated benoxacor were also modeled to experience a significant decline in likelihood of successful emergence (~50%). Males and females exposed to the mixture (200 mg/kg) had the lowest likelihood (0% and 3.4% compared to the solvent control group) in successful emergence chances of all (Table 4). We observed a significant effect of the mixture at lower concentrations (0.02 and 0.2 mg/kg) on the probability of a females' successful emergence. These decreases (~56 and 36% likely compared to the control) were close to the reduction seen in females exposed to benoxacor alone at the high level.

DISCUSSION

Each chemical exposure at the 100 mg/kg (200 mg/kg mixture) concentration was toxic to *C. riparius*, significantly affecting survival, with the

412 mixture exposure resulting in the most severe decline in survival, followed by
413 benoxacor, mono-chlorinated benoxacor, and S-metolachlor. Our experiments
414 demonstrated that benoxacor and its transformation product are likely more toxic
415 than S-metolachlor to *C. riparius*, based on the number of responses affected and to
416 what severity at various concentrations, depending on the endpoint. Our data
417 demonstrated the likely dose-response curve for *C. riparius* survival lies between
418 the 10 to 100 mg/kg range for all four chemical types, which likely also differ in
419 slope. Although this range is not anticipated to represent an environmentally-
420 relevant exposure level, our study demonstrates the relative toxicity of the safener
421 and its transformation product to S-metolachlor.

422 Given that these chemicals can affect emergence rate and even more so the
423 hazard of not emerging successfully in males and females, it is possible that the two
424 sexes could be affected to different extents, which would have the potential to
425 disrupt the chances of successful copulation within a population. Statistically
426 significant effects on emergence rate only occurred in select treatment groups:
427 males and females who were exposed to high concentrations benoxacor, and males
428 and females exposed to high concentrations of the mixture. Although statistically
429 significant, the biological significance of the small decreases in emergence rate
430 observed in the high treatment exposure is unclear. The ability for these chemicals
431 to affect emergence rate is, however, evidence of their ability to affect sublethal
432 endpoints in *C. riparius*, which is particularly important given that *C. riparius* adults
433 depend on simultaneous male and female emergence in order to copulate in the
434 field [17].

A clear decline in adult male (but not female) dry body mass was apparent in high concentrations of S-metolachlor. This trend was not apparent in any of the other chemical treatments. The potential for S-metolachlor to behave as an endocrine disruptor has been demonstrated in prepubertal male Wistar rats [28], however following a Tier I screening assay directed by USEPA's Endocrine screening program, S-metolachlor was not recommended for further Tier II studies on mammalian organisms, nor wildlife (including aquatic organisms) due to a demonstrated lack of evidence for interaction with estrogen, androgen or thyroid pathways [29].

Females exposed to the mixture of S-metolachlor and benoxacor are susceptible to a decrease in the likelihood of successfully emerging when exposed to lower concentrations (0.02-0.2 mg/kg) as well as the high concentration (200 mg/kg). Females exposed to benoxacor at 1 mg/kg, and mono-chlorinated benoxacor at 0.1 mg/kg, also demonstrated significant decreases in their likelihoods of successfully emerging. Meanwhile, males only responded in significant decreases in their likelihoods of successfully emerging when exposed to the high concentration of each chemical.

Jin-Clark et al. (2008) investigated the effect of metolachlor on major detoxification enzymes in another common *Chironomus* species, *C. tentans* [30]. At 1000 µg/L, metolachlor reduced acetylcholinesterase (AChE) activity by 27.6% in the treated midges [30]. Metolachlor also reduced protein production by 3.2-fold, which was associated with a 2.8-fold reduction of cytochrome P450 O-deethylation total activity, and 1.4 – 1.7-fold reductions of GST total activities in the treated

458 midges [30]. These reductions in total activities of major detoxification enzymes
459 may impede detoxification of other environmental contaminants (e.g. chlorpyrifos)
460 and increase the midges' susceptibility to toxins [30]. Since S-metolachlor is
461 commonly paired with benoxacor, and they share similar physical/chemical
462 properties (Table 1), it is plausible S-metolachlor could also inhibit the
463 detoxification of benoxacor in *C. riparius* under field scenarios.
464 Larvae in microcosms containing the 100 mg/kg levels of benoxacor alone or
465 in mixture, or in microcosms with 100 mg/kg of the degradation product mono-
466 chlorinated benoxacor, exhibited abnormal behavioral changes, which were noted
467 in daily observations (Supplemental Data, Table 27). Larvae in high concentrations
468 of benoxacor and/or the mixture were observed sporadically writhing on the
469 sediment surface, lethargically lying on the sediment surface, or alternating
470 behavior between the two. Organisms writhing on the sediment surface or lying
471 lethargically were seen in groups of up to 12; these organisms behaved this way for
472 up to a few days before dying. Such behavior would be characteristic of a toxin
473 interacting with the organisms' nervous systems [31]. Similar behavior has been
474 observed in *Chironomus tentans* following exposure to perfluorooctane sulfonic acid
475 [32], however our larvae did not lose their red coloration until the very point of
476 death. This suggests benoxacor/monochlorinated benoxacor were stressing the
477 nervous system rather than the respiratory system as suggested by Macdonald et al.
478 (2004). Larvae in the 100 mg/kg mono-chlorinated benoxacor replicates were
479 observed with posterior ends resting just above the sediment surface (larva still
480 partially within its sediment burrow). Both behaviors would increase the

organism's vulnerability to predators. These abnormal behaviors may prove useful indicators for determining the mode of action of benoxacor and related dichloroacetamide safeners in non-target aquatic and terrestrial animals.

Benoxacor is capable of transforming via surface-mediated reductive dechlorination in slurries containing iron (hydr)oxide + ferrous iron [5]. In the *C. riparius* exposure microcosms, which were simply larger versions of the spike recovery models (plus *C. riparius*), benoxacor could have been transformed into mono-chlorinated or des-chlorinated benoxacor. The standard kaolin or peat based sediment commonly used in sediment toxicity tests would not provide an appropriate environment to study the degradation of this class of safeners, since such an iron-deficient environment would not support the transformation of benoxacor inferred within our microcosms (Figure 3). The benoxacor half-life we estimated in both aqueous and sediment phases within the sediment microcosm, appears to be shorter than that published for anaerobic soil (70 days) [7]. Some of the transformation we observed may also have been due to biotransformation by the diversity of microorganisms present from the field-collected sediment held in the laboratory, as well as the chemical microenvironment in this sediment.

The likelihood of exposure to the benoxacor is not negligible for fresh-water organisms living in surface water bodies, given prior data on environmental concentrations of S-metolachlor in surface and groundwater. S-metolachlor is considered a common contaminant in surface and groundwater [33] frequently detected in proximity to areas where S-metolachlor is used on crops [33]. S-metolachlor has been routinely detected at 11.5 ug/L in surface water and 0.25 ug/L

504 in groundwater [34]; although the maximum concentration detected has reached
505 1.38 ppm in surface water [34, 35] this level is still low relative to the LOEC
506 determined in this study. S-metolachlor is one of the two most commonly found
507 pesticides in shallow groundwater within agricultural areas [33]. When unique
508 mixtures were analyzed, metolachlor and atrazine were detected together 77% of
509 the time and in 15% of samples, from agricultural surface and groundwater,
510 respectively. More than 30 percent of 5-compound unique mixtures in agricultural
511 streams included metolachlor [33]. Given such a wide distribution in natural
512 waters, we surmise that benoxacor, as a component of commercial herbicide
513 formulations, should have a comparably extensive occurrence, though little/no data
514 on its concentrations in nature is available.
515 The partitioning of benoxacor in both the aqueous and sedimentary phases,
516 as well as its half-life, have important consequences for ecotoxicological impacts of
517 benoxacor on both pelagic and benthic organisms with similar life histories. This
518 study recommends measurement of the concentrations of benoxacor (and other
519 dichloroacetamide safeners) in surface and groundwater of agricultural areas to
520 assess potential risk. Given that *C. riparius* is a pollution tolerant test organism [17],
521 and that behavioral and physiological responses to these chemicals in *C. riparius*
522 occurred between 10-100 mg/kg sediment, it is likely that more sensitive organisms
523 would be affected at lower concentrations. Our observations warrant further
524 examination of benoxacor and its degradation products on sublethal behavioral
525 effects on the larval-to-pupal life-cycle stages of *C. riparius* at lower levels (1-50
526 mg/kg), as well as aquatic toxicity studies of the other dichloroacetamide safeners

(dichlormid, furilazole, AD-67) in environmentally relevant sediment-based test conditions.

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684

FIGURE TITLES AND LEGENDS

1. Reductive dehalogenation of benoxacor into its transformation products, monochlorobenoxacor and deschlorobenoxacor, in the presence of Fe(II)-amended iron (hydr) oxide, adapted from reference 4.

2. Experimental design and randomization of exposure microcosms in the Urban Environmental Biogeochemical Laboratory at Towson University.

3. Aqueous-phase benoxacor concentrations as a function of time in selected microcosm components. In aqueous-phase only systems (open symbols), benoxacor was added as a methanolic spike to yield a nominal concentration of 80 μM. In microcosms containing solids (filled symbols), sand was amended with benoxacor at a level sufficient to yield a nominal aqueous-phase concentration of 80 μM (assuming complete desorption). Lines denote kinetic model fits to the data (see Table 3).

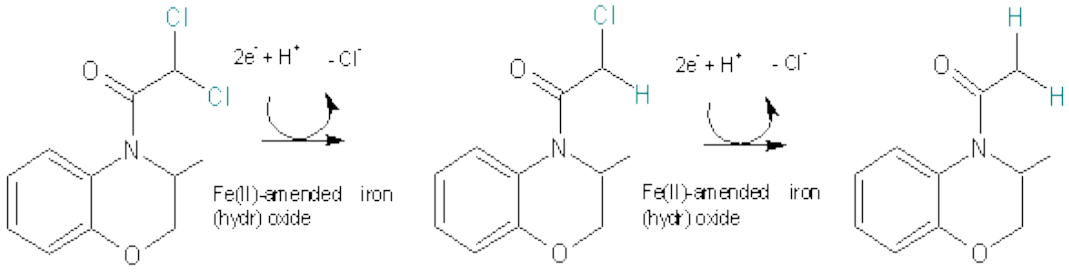
4. Mean total adult emergence ratio of larval organisms exposed to benoxacor (A), monochlorinated benoxacor (B), S-metolachlor (C), and the mixture (D). Error bars represent standard deviation of the mean.

5. Mean emergence rate of males exposed to benoxacor (A), females (B), males exposed to the mixture (C), and females exposed to the mixture (D). Error bars represent standard deviation of the mean.

6. Mean dry body weight (g) of males exposed to S-metolachlor. Error bars represent standard deviation of the mean.

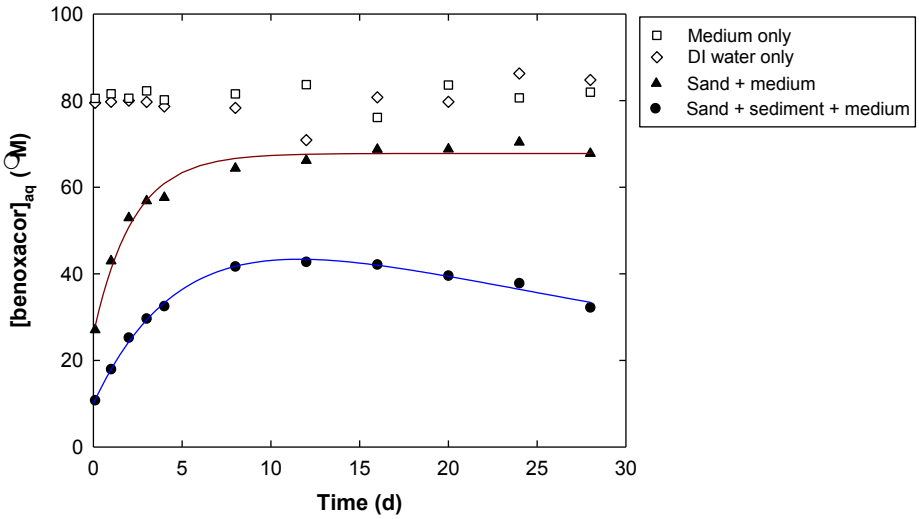
7. Time-to-healthy emergence curves of males and females exposed to benoxacor (A, B), mono-chlorinated benoxacor (C, D), S-metolachlor (E, F), and the mixture (G, H). Blue = solvent control group; Cyan = 0.02 mg/kg group; Green = 0.1 or 0.2 mg/kg; Orange = 1 mg/kg group; Red = 100 mg/kg (200 mg/kg mixture) group; Gray = non-significant difference from solvent control group.

1.

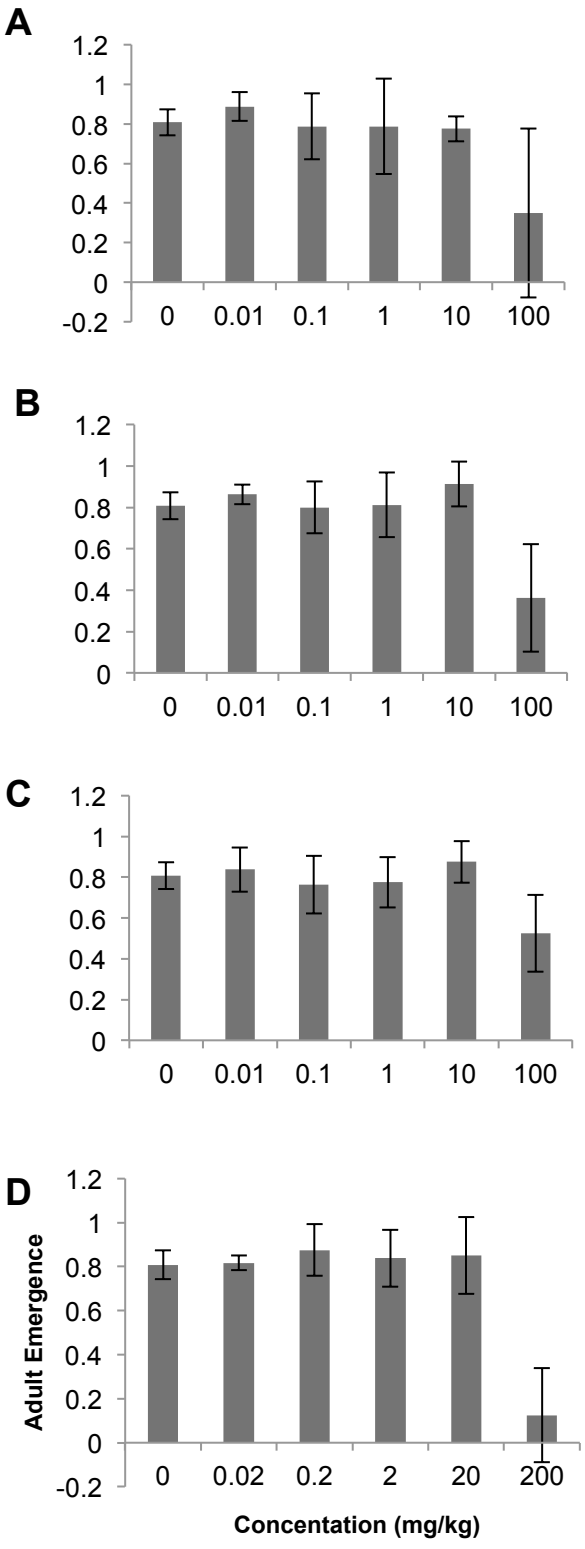

Cl
O
Cl
N
O
2e⁻ + H⁺
- Cl⁻
Fe(II)-amended iron (hydr) oxide
Cl
O
H
N
O
2e⁻ + H⁺
- Cl⁻
Fe(II)-amended iron (hydr) oxide
H
O
H
N
O

2.

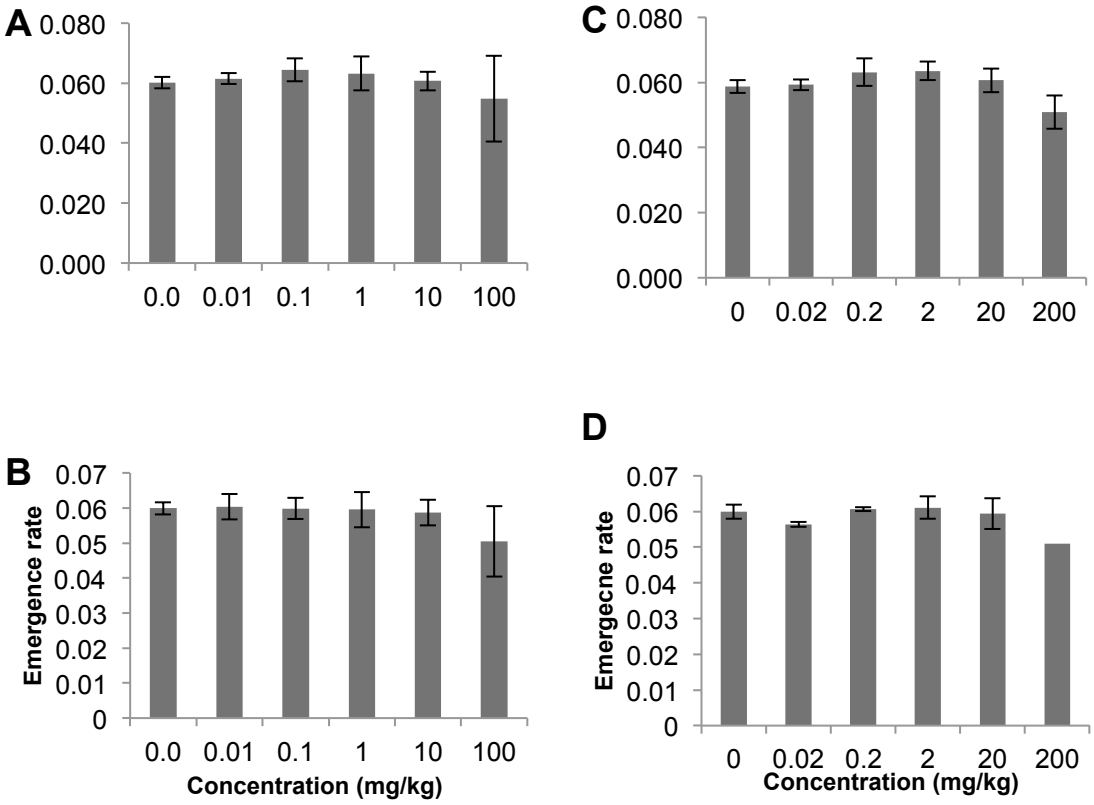
3.


100
80
60
40
20
0
0
5
10
15
20
25
30
[benoxacor]aq (μM)
Time (d)
Medium only
DI water only
Sand + medium
Sand + sediment + medium

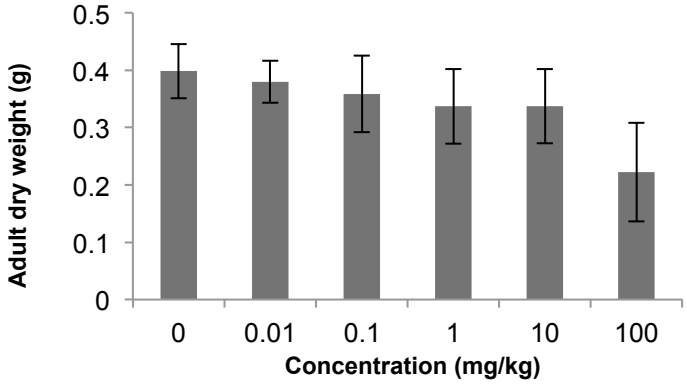
4.


A
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
0.01
0.1
1
10
100
B
1.2
1
0.8
0.6
0.4
0.2
0
0
0.01
0.1
1
10
100
C
1.2
1
0.8
0.6
0.4
0.2
0
0
0.01
0.1
1
10
100
D
Adult Emergence
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
0.02
0.2
2
20
200
Concentation (mg/kg)

5.


A
0.080
0.060
0.040
0.020
0.000
0.0 0.01 0.1 1 10 100
C
0.080
0.060
0.040
0.020
0.000
0 0.02 0.2 2 20 200
B
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Emergence rate
0.0 0.01 0.1 1 10 100
Concentration (mg/kg)
D
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Emergecne rate
0 0.02 0.2 2 20 200
Concentration (mg/kg)

6.


0.5
0.4
0.3
0.2
0.1
0
Adult dry weight (g)
0
0.01
0.1
1
10
100
Concentration (mg/kg)

7.

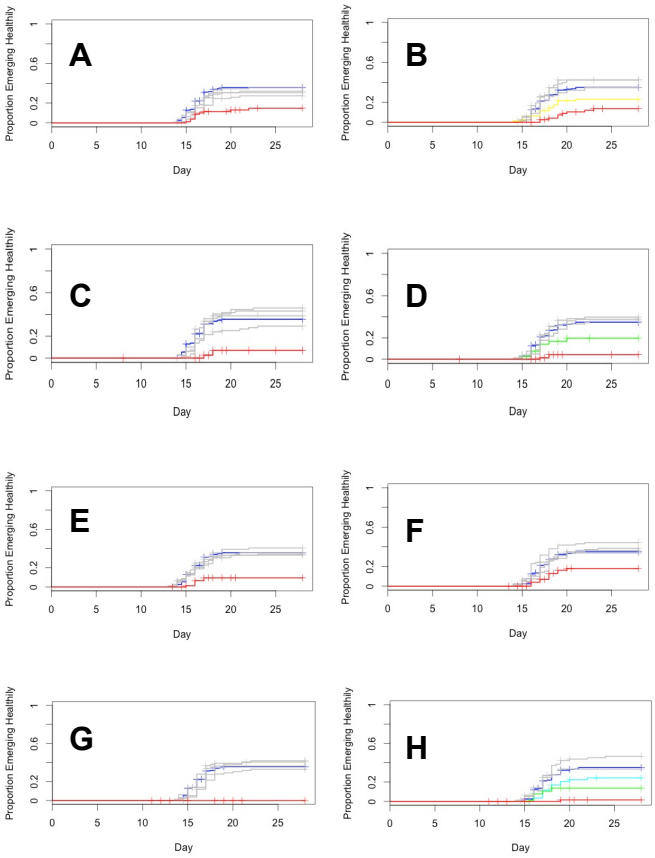
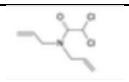
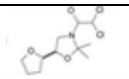
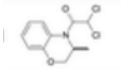
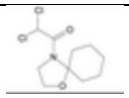
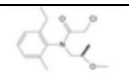
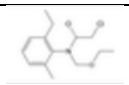

A
B
C
D
E
F
G
H
Proportion Emerging Healthily
Day
0
5
10
15
20
25
0.2
0.6
1

Table 1. Physical and chemical properties of dichloroacetamide safeners and herbicidal co-formulants[a]

Common Name	Additional Identifier	Structure	Log K_{ow}[b]	C_w^{satb}	Log K_{aw}[b]	Aerobic DT_{50}[c]	Anaerobic DT_{50}[c]	Typical Herbicidal Coformulant
Dichlormid	R-25788		1.84	1070	-4.87	8 days	n/avail	Acetochlor
Furilazole	MON 13900		2.12	254	-8.42	13 days[38]	n/avail	Acetochlor
Benoxacor	CGA-154281		2.70	103	-5.51	49 days[33]	70 days[33]	Metolachlor
AD-67	MON 4660		3.19	43	-7.29	18 days[37]	n/avail	Acetochlor
Metolachlor	---		2.90	51	-6.43	26 days[35]	37 days[35]	---
Acetochlor	---		3.03	47	-6.04	11 days[39]	19 days[39]	---

[a]Table adapted from Sivey et al. [4]

[b]Octanol-water (K_{ow}) and air-water (K_{aw}) partition coefficients and water solubility (C_w^{sat}, 25 °C) data from reference [36].

[c]DT_{50} denotes the median dissipation half-life of the parent compound in soil.

Table 2. Definitive experiments' dosing stock concentrations confirmed by HPLC

Chemical	Treatment Level	Stock Concentration (mg/mL)
BN	1	0.0049
	2	0.0131
	3	0.124
	4	1.37
	5	11.9
BNMCL	1	0.0053
	2	0.0163
	3	0.110
	4	0.795
	5	12.6
SM	1	0.0021
	2	0.0128
	3	0.127
	4	1.80
	5	12.6
MIX (BN)	1	0.0017
	2	0.015

	3	0.122
	4	0.994
	5	9.72
MIX (SM)	1	n.d.
	2	0.0102
	3	0.133
	4	0.908
	5	9.58

BN = Benoxacor; BNMCL = Monochlorinated benoxacor; SM = S-metolachlor; MIX = Mixture; n.d. = not determined

Table 3. Kinetic Models and Rate Constants Associated with the Dissolution and Persistence Data Shown in Figure 3 [a]

System	**Sand + medium**	**Sand + sediment + medium**
Best-fit kinetic model [b]	$\frac{d[\mathrm{BN}]_{\mathrm{aq}}}{d\mathrm{t}} = k_{des}[\mathrm{BN}]_{\mathrm{s}} - k_s[\mathrm{BN}]_{\mathrm{aq}}$ $\frac{d[\mathrm{BN}]_{\mathrm{s}}}{d\mathrm{t}} = -k_{des}[\mathrm{BN}]_{\mathrm{s}} + k_s[\mathrm{BN}]_{\mathrm{aq}}$	$\frac{d[\mathrm{BN}]_{\mathrm{aq}}}{d\mathrm{t}} = k_{des}[\mathrm{BN}]_{\mathrm{s}} - k_s[\mathrm{BN}]_{\mathrm{aq}}$ $\frac{d[\mathrm{BN}]_{\mathrm{s}}}{d\mathrm{t}} = -k_{des}[\mathrm{BN}]_{\mathrm{s}} + k_s[\mathrm{BN}]_{\mathrm{aq}} - k_t[\mathrm{BN}]_{\mathrm{s}}$
k_{des} (d^{-1})	0.38 ± 0.07	0.148 ± 0.017
k_s (d^{-1})	0.07 ± 0.02	0.024 ± 0.007
k_t (d^{-1})	not applicable	0.06 ± 0.03

[a] Rate constants include desorption of benoxacor (BN) from solids (k_{des}), sorption to solids (k_s), and transformation (k_t). Uncertainties denote 95% confidence intervals.

[b] $[BN]_{aq}$ and $[BN]_s$ denote concentrations (in μM) of BN in the aqueous phase and sorbed to solids, respectively. Assumes transformation of BN is negligible in the aqueous phase and when adsorbed onto sand.

Table 4. Significant cox proportional hazard model results[a]

Group	Treatment (mg/kg)	Coef	Exp(coef)	SE(coef)	Z	Pr(>|z|)
BN males	100	-1.060	0.346	0.321	-3.300	9.67e-04**
BN females	1	-0.554	0.574	0.268	-2.068	0.0386*
BN females	100	-1.241	0.288	0.351	-3.528	4.18e-04***
BNMCL males	100	-1.944	0.142	0.460	-4.221	2.43e-05***
BNMCL females	0.1	-0.687	0.503	0.282	-2.436	0.0149
BNMCL females	100	-2.365	0.093	0.588	-4.020	5.83e-05***
SM males	100	-1.461	0.231	0.393	-3.712	2.06e-04***
SM females	100	-0.908	0.403	0.310	-2.929	3.402e-03**
Mix males	200	-1.822e+01	1.221e-08	1.673e+03	-0.011[b]	0.991[b]
Mix females	0.02	-0.576	0.561	0.294	-1.962	0.04979*
	0.2	-1.032	0.356	0.336	-3.070	0.00214**
	200	-3.378	0.034	1.006	-3.356	7.9e-04**

[a]The cox proportional hazards model data are summarized as treatment level (mg/kg) larvae were exposed to, the hazard coefficient and it's exponentiated form, Z value and p-value.

[b]MLE (maximum likelihood estimate) is infinity because one of the groups had no events, and log likelihood converged. In this case the Wald statistic (Z) should be ignored but the likelihood ratio and score tests are valid.

** $p < 0.005$.

*** $p < 0.0005$.

BN = Benoxacor; BNMCL = Monochlorinated benoxacor; SM = S-metolachlor; MIX = Mixture.

1a. Emergence ratio summary from rangefinder 1.[a]

Treatment (mg a.i./kg)	Average % Males from Those Emerged	Average % Females From Those Emerged	Average % Emerged All Adults
Negative Control	47%	53%	85%
Solvent Control	57%	43%	88%
0.01	47%	53%	85%
0.1	47%	53%	85%
1	40%	60%	63%
10	53%	47%	85%
100	41%	59%	68%

[a]Larval organisms were exposed to nominal concentrations of benoxacor ranging from 0.01 mg a.i./kg to 100 mg a.i./kg.

1b. Emergence ratio summary from rangefinder 2.[a]

Treatment (µg a.i./kg)	Average % Males from Those Emerged	Average % Females From Those Emerged	Average % Emerged All Adults
NC	44%	56%	85%
SC	60%	40%	100%
0.05	51%	49%	88%
0.5	56%	44%	85%
7.8	44%	56%	80%
100	49%	51%	93%
1000	50%	50%	90%

[a]Larval organisms were exposed to nominal concentrations of benoxacor ranging from 0.01 ug a.i./kg to 1000 ug a.i./kg.

1c. Emergence ratio summary from rangefinder 3.[a]

Treatment (µg a.i./kg)	Average % Males from Those Emerged	Average % Females From Those Emerged	Average % Emerged All Adults
NC	54%	46%	93%
SC	48%	53%	100%
0.09	31%	69%	80%
0.89	50%	50%	90%
9.4	44%	56%	90%
100	66%	34%	88%
1000	52%	48%	78%

[a]Larval organisms were exposed to nominal concentrations of S-metolachor ranging from 0.01 ug a.i./kg to 1000 ug a.i./kg.

2a. Stock (measured using a glass 2 mL pipette) added to sand (5% dry weight of the corresponding sediment batch); recorded mass (g) of stock spiked to sand, recorded using digital balance and subsequently calculated amount of methanolic stock (mL) added to sand; calculated amount of test substance added by mass (mg) from HPLC confirmed stock concentrations, and the calculated nominal concentration of that batch of spiked sediment based on dry batch weight (196 g and 171.5 g, block 1 and 2).

	Treatment level	Recorded stock added (g)	Stock added (mL methanol based on mass)	Final test substance added by weight (mg)	**Final nominal concentration of batch**
Block 1: BN	1	1.4	1.78	0.01	0.044
	2	1.4	1.8	0.02	0.109
	3	1.3	1.78	0.20	1.038
	4	1.3	1.75	2.25	11.47
	5	1.5	1.88	22.54	115.01
Block 1: BNMCL	1	1.3	1.83	0.01	0.044
	2	1.4	1.85	0.03	0.147
	3	1.4	1.83	0.19	0.992
	4	1.4	1.95	1.41	7.171
	5	1.5	1.92	23.87	121.784
Block 1: SM	1	1.3	1.81	0.00	0.0175
	2	1.3	1.8	0.02	0.107
	3	1.3	1.78	0.21	1.06
	4	1.3	1.84	2.96	15.07
	5	1.4	1.97	22.28	113.66
Block 1: MIX	1	1.4	1.88	0.0018[a]	0.0247[a]
	2	1.1	1.44	0.04	0.178
	3	1.3	1.74	0.42	2.13
	4	1.3	1.73	3.12	15.9
	5	1.5	1.98	36.56	186.5
Block 2: BN	1	1.22	1.56	0.01	0.044
	2	1.24	1.57	0.02	0.110
	3	1.22	1.56	0.19	1.114
	4	1.17	1.53	2.02	11.80
	5	1.32	1.65	19.84	115.67
Block 2: BNMCL	1	1.22	1.57	0.01	0.0476
	2	1.24	1.6	0.03	0.148
	3	1.32	1.7	0.18	1.069
	4	1.33	1.7	1.34	7.786
	5	1.32	1.67	21.01	122.47

Block 2: SM	1	1.23	1.59	0.00	0.019
	2	1.14	1.57	0.02	0.107
	3	1.13	1.56	0.18	1.0568
	4	1.23	1.61	2.80	16.304
	5	1.31	1.73	20.85	121.552
Block 2: MIX	1	1.11	1.65	0.00105[a]	0.022[a]
	2	0.95	1.26	0.0302	0.176
	3	1.16	1.52	0.3735	2.178
	4	1.12	1.51	2.6903	15.687
	5	1.32	1.74	32.174	187.608

[a]Low concentration of S-metolachlor was not confirmed by HPLC and the gravimetrically calculated stock concentration of 0.00104 mg/mL was used for sediment concentration calculations.

2b.

Chemical	Aimed-for concentration (mg/kg)	Median nominal sediment concentration between both blocks (mg/kg)
Benoxacor	0.01	0.04
	0.1	0.11
	1	1.08
	10	11.64
	100	115.35
Mono-chlorinated benoxacor	0.01	0.05
	0.1	0.15
	1	1.03
	10	7.48
	100	122.13
S-metolachlor	0.01	0.02
	0.1	0.11
	1	1.06
	10	15.69
	100	117.61
Mixture	0.01	0.023[a]
	0.1	0.18
	1	2.16
	10	15.81
	100	187.08

[a]Low concentration of S-metolachlor was not confirmed by HPLC and the gravimetrically calculated stock concentration of 0.00104 mg/mL was used for sediment concentration calculations.

3. Wet weight (g) of spiked sediment layer added to each experimental replicate.

	Treatment level mg/kg	Replicate	Sediment added to replicate (g)
Block 1: BN	0.01	A	170.1
	0.01	B	170.0
	0.1	A	170.1
	0.1	B	170.0
	1	A	170.2
	1	B	170.1
	10	A	170.0
	10	B	170.1
	100	A	170.2
	100	B	170.1
Block 1: BNMCL	0.01	A	170.0
	0.01	B	170.2
	0.1	A	170.2
	0.1	B	170.0
	1	A	170.0
	1	B	170.0
	10	A	170.2
	10	B	170.2
	100	A	170.0
	100	B	170.0
Block 1: SM	0.01	A	170.0
	0.01	B	170.2
	0.1	A	170.1
	0.1	B	170.0
	1	A	170.1
	1	B	170.3
	10	A	170.0
	10	B	170.0
	100	A	170.0
	100	B	170.0
Block 1: MIX	0.01	A	170.1
	0.01	B	170.3
	0.1	A	170.0
	0.1	B	170.1
	1	A	170.0
	1	B	170.0
	10	A	170.3
	10	B	170.2
	100	A	170.0

	100	B	170.1
Block 1: NC		A	170.0
		B	170.0
		C	170.0
		D	170.1
		E	170.2
		F	170.3
		G	170.3
		H	170.1
Block 1: SC		A	170.0
		B	170.0
		C	170.1
		D	170.0
		E	170.0
		F	170.3
		G	170.3
		H	170.2
Block 2: BN	0.01	A	170.00
	0.01	B	170.93
	0.1	A	170.74
	0.1	B	170.60
	1	A	169.79
	1	B	1171.29
	10	A	171.03
	10	B	169.31
	100	A	170.48
	100	B	171.91
Block 2: BNMCL	0.01	A	170.71
	0.01	B	171.59
	0.1	A	173.05
	0.1	B	169.80
	1	A	170.10
	1	B	172.50
	10	A	172.90
	10	B	170.08
	100	A	171.42
	100	B	172.61
Block 2: SM	0.01	A	171.27
	0.01	B	170.83
	0.1	A	169.94
	0.1	B	170.52
	1	A	170.71
	1	B	171.23
	10	A	170.75

	10	B	170.31
	100	A	170.31
	100	B	169.18
Block 2: MIX	0.01	A	169.56
	0.01	B	170.72
	0.1	A	170.38
	0.1	B	170.73
	1	A	169.05
	1	B	169.40
	10	A	170.93
	10	B	170.97
	100	A	170.32
	100	B	169.55
Block 2: NC		A	169.71
		B	171.43
		C	170.09
Block 2: SC		A	169.87
		B	171.64
		C	172.09
		D	170.18

4. Water chemistry measurements: Block 1

Treatment (BN)	Day 1	Day 4	Day 7	Day 10	Day 14	Day 18	Day 22	Day 28
Temperature (°C)								
0.01 mg/kg	21	21	20	21	19	18.6	20.3	20.3
0.1 mg/kg	21	20	20	21	18.6	18.7	20.2	20.1
1 mg/kg	21	21	21	21	18.2	18.8	20.2	19.9
10 mg/kg	21	20	20	21	18.2	18.6	20	19.7
100 mg/kg	21	21	21	20	18.2	18.3	19.9	19.5
DO (%)								
0.01 mg/kg	102.7	91.4	94.3	100.7	99.6	93.7	91.8	86.6
0.1 mg/kg	104.4	92.6	95.4	102.6	102.3	93.4	92.8	86.4
1 mg/kg	106.4	93.2	96.8	102.9	103.1	64.5	91.9	85.5
10 mg/kg	105.1	93.2	97.5	101.8	103.3	93.6	93.3	81.3
100 mg/kg	106.8	94.6	98.2	102.8	102.2	95.2	93.4	89.2
pH								
0.01 mg/kg	7.8	7.7	8.2	8.2	8.8	8.6	8.4	8.4
0.1 mg/kg	7.8	7.8	8.2	8.1	8.8	8.8	8.5	8.4
1 mg/kg	7.8	7.8	8.2	8.1	8.8	8.9	8.5	8.3
10 mg/kg	7.8	7.9	8.2	8.1	8.8	8.7	8.5	8.3
100 mg/kg	7.8	7.9	8.2	8.1	8.8	8.3	8.5	8.3
Treatment (BNMCL)	Day 1	Day 4	Day 7	Day 10	Day 14	Day 18	Day 22	Day 28
Temperature (°C)								
0.01 mg/kg	21	20	20	21	18.8	18.3	19.8	20.2
0.1 mg/kg	20	20	20	21	18.8	18.6	19.7	20.1
1 mg/kg	20	21	20	21	18.8	18.8	19.6	19.6
10 mg/kg	20	20	20	20	18.9	18.8	19.6	20.2
100 mg/kg	20	20	20	21	18.9	18.9	19.6	19.3
DO (%)								
0.01 mg/kg	106	104.2	94.3	105.2	100.9	98	92	92.5
0.1 mg/kg	104.8	105	95.8	106.3	100.6	93.6	93.1	93.7
1 mg/kg	103.5	104.5	94.9	105.2	101.8	92	93.3	92.4
10 mg/kg	101.9	102.8	93.7	104	98.7	89.5	95.4	91.9
100 mg/kg	102.3	104.6	95.5	106.5	101.1	86.9	95.5	92.6
pH								
0.01 mg/kg	7.8	7.7	8.4	7.9	8.7	8.7	8.6	8.5
0.1 mg/kg	7.8	7.8	8.4	8	8.7	8.7	8.6	8.5
1 mg/kg	7.8	7.9	8.4	8	8.7	8.3	8.6	8.4
10 mg/kg	7.9	7.9	8.4	8	8.6	8.4	8.6	8.5

100 mg/kg	7.9	7.9	8.4	8.1	8.6	8.2	8.6	8.5
Treatment (SM)	Day 1	Day 4	Day 7	Day 10	Day 14	Day 18	Day 22	Day 28
Temperature (°C)								
0.01 mg/kg	21	21	21	21	19.8	19.8	19.5	20.2
0.1 mg/kg	21	21	21	21	19.8	19.6	19.5	20.2
1 mg/kg	21	20	21	20	19.9	19.8	19.5	21
10 mg/kg	21	20	21	20	19.8	19.8	19.5	19.8
100 mg/kg	21	21	21	20	19.8	19.8	19.5	19.6
DO (%)								
0.01 mg/kg	103.6	103.2	93.6	103	93	84.5	92.7	92.3
0.1 mg/kg	103.6	103.3	92.8	102.7	92.2	85.1	94.9	92.5
1 mg/kg	104.2	104.2	94.3	103.4	94.4	88.1	96.2	93.1
10 mg/kg	106.5	103.8	94	103.4	94.4	87.5	96	95.1
100 mg/kg	106	102.7	94.3	101.9	94.5	87	95.4	92.4
pH								
0.01 mg/kg	7.7	8.3	8.4	8	8.6	8.6	8.7	8.6
0.1 mg/kg	7.7	8.3	8.3	8	8.5	8.7	8.7	8.6
1 mg/kg	7.8	8.3	8.3	8.1	8.5	8.8	8.7	8.6
10 mg/kg	7.8	8.3	8.3	8.1	8.5	8.6	8.7	8.6
100 mg/kg	7.8	8.3	8.3	8.1	8.5	8.5	8.7	8.6
Treatment (MIX)	Day 1	Day 4	Day 7	Day 10	Day 14	Day 18	Day 22	Day 28
Temperature (°C)								
0.02 mg/kg	20	20	20	20	19.8	20	19.7	19.3
0.2 mg/kg	20	20	20	20	19.8	20	19.8	19.3
2 mg/kg	21	20	20	21	19.8	20	19.9	19.4
20 mg/kg	21	20	20	21	19.8	19.9	19.9	19.4
200 mg/kg	21	20	20	21	19.8	20	19.9	19.4
DO (%)								
0.02 mg/kg	104.3	74.8	94.7	100	94	87.6	94.7	92.4
0.2 mg/kg	105.5	78.3	96.4	103.4	94.4	87.5	95.9	90
2 mg/kg	104.8	100.2	96	101	92.4	81.2	93.7	91.2
20 mg/kg	104.8	99.2	95.3	102.4	93.4	85	92.8	91.2
200 mg/kg	102.9	98.8	94.5	101.6	92.8	86.8	94	90.4
pH								
0.02 mg/kg	7.7	8.2	8.4	8.1	8.6	8.8	8.7	8.6
0.2 mg/kg	7.7	8.3	8.4	8.1	8.6	8.8	8.7	8.6
2 mg/kg	7.8	8.3	8.4	8.1	8.6	8.5	8.7	8.6
20 mg/kg	7.8	8.3	8.4	8.1	8.6	8.6	8.7	8.6

200 mg/kg	7.9	8.3	8.4	8.1	8.5	8.3	8.7	8.6
Treatment (NC)	Day 1	Day 4	Day 7	Day 10	Day 14	Day 18	Day 22	Day 28
Temperature (°C)	21	21	20	21	19.3	18.8	20.6	21.1
	21	20	20	21	18.7	18.3	19.8	19.4
	20	21	21	20	19.9	19.8	19.6	19.8
	20	20	20	21	19.8	19.9	19.6	20.3
DO (%)	97.7	90.1	92	97.3	99	94	89	87.7
	106.2	104.3	95.9	102.7	100.5	93.1	94.2	88
	101.4	104.5	94.2	102.2	94	90.4	95.3	93.7
	105	100.6	97.3	102.8	95.4	86.9	96	91.4
pH	7.7	7.6	8	8.3	8.9	8.5	8.2	8.4
	7.7	7.6	8.4	7.8	8.8	8.6	8.5	8.3
	7.5	7.8	8.4	7.8	8.6	8.6	8.6	8.6
	7.5	8.2	8.5	8.1	8.6	8.7	8.7	8.6
Treatment (SC)	Day 1	Day 4	Day 7	Day 10	Day 14	Day 18	Day 22	Day 28
Temperature	21	21	20	21	19.2	18.7	20.5	21
	21	20	20	21	18.8	18.3	19.8	19.9
	21	21	21	20	19.9	19.5	19.5	19.9
	20	20	20	21	19.8	20	19.7	19.2
DO (%)	104.7	91.8	92.2	103.5	99.2	96	91.5	86
	105.3	105.2	96.1	104.3	100.6	97.1	93.5	92.2
	105	104.8	95.6	103.7	96	89.6	96.3	93.9
	105	98.8	96	102.1	92.4	86.4	94.2	91.5
pH	7.7	7.7	8.1	8.2	8.8	8.7	8.3	8.4
	7.7	7.7	8.4	7.9	8.8	8.7	8.6	8.4
	7.6	8.2	8.4	7.9	8.6	8.7	8.7	8.6
	7.5	8.3	8.4	8.1	8.6	8.7	8.7	8.6

5. Water chemistry measurements: Block 2

Treatment (BN)	Day 1	Day 8	Day 13	Day 20	Day 28
Temperature (°C)					
0.01 mg/kg	19.9	19.5	19.3	20.3	19.4
0.1 mg/kg	19.6	19.1	19.3	19.8	20
1 mg/kg	19.7	18.9	19.2	19.6	17
10 mg/kg	19.3	19.3	19.2	20.2	16.9
100 mg/kg	19.1	19.7	19	20	17.4
DO (%)					
0.01 mg/kg	93.2	93.7	93.6	87.3	98
0.1 mg/kg	93	93.5	91.5	91.1	96
1 mg/kg	92.3	99.8	93.5	90.2	114.6
10 mg/kg	93.1	94.5	92.9	88.8	112.9
100 mg/kg	94.2	95.6	93	88.6	113.5
pH					
0.01 mg/kg	7	7	7.5	7.7	8.2
0.1 mg/kg	7	7	7.6	7.7	8.1
1 mg/kg	7.1	7.1	7.6	7.7	8.3
10 mg/kg	7.1	7.1	7.6	7.8	8.2
100 mg/kg	7.1	7.1	7.6	7.8	8.3
Treatment (BNMCL)	Day 1	Day 8	Day 13	Day 20	Day 28
Temperature (°C)					
0.01 mg/kg	19	18.5	18.8	20	17.4
0.1 mg/kg	18.9	18.9	19	20.4	17.5
1 mg/kg	18.8	19.4	18.6	19.3	17.3
10 mg/kg	18.8	19.1	18.5	19.5	17.3
100 mg/kg	18.7	20	18.4	19.1	17.8
DO (%)					
0.01 mg/kg	96.8	88.9	90.3	89.8	111.2
0.1 mg/kg	95.7	98.7	87.5	75.4	111.4
1 mg/kg	95.5	97.9	92.8	90.9	98.6
10 mg/kg	95.3	99.2	87.5	93.5	104.3
100 mg/kg	95.5	94.3	94	92.2	101.1
pH					
0.01 mg/kg	7.2	7.1	7.6	7.8	8.3
0.1 mg/kg	7.2	7.2	7.6	7.8	8.3
1 mg/kg	7.3	7.1	7.7	7.8	8.3
10 mg/kg	7.3	7.2	7.6	7.8	8.3
100 mg/kg	7.3	7.2	7.6	7.8	8.3

Treatment (SM)	Day 1	Day 8	Day 13	Day 20	Day 28
Temperature (°C)					
0.01 mg/kg	18.6	20.1	18.4	20.2	17.3
0.1 mg/kg	18.6	19.6	18.4	20.2	16.8
1 mg/kg	18.5	19.8	18.4	20.3	16.8
10 mg/kg	18.5	19.9	18.5	19.7	16.8
100 mg/kg	18.5	18.9	18.5	19.6	16.9
DO (%)					
0.01 mg/kg	95.5	96	97.3	91.3	110.5
0.1 mg/kg	99.6	96.3	98	94.4	113.4
1 mg/kg	98.8	95.8	96.2	89.5	116.3
10 mg/kg	99.7	94.7	96.5	87.6	109.5
100 mg/kg	98.1	93.1	95.2	79.9	113
pH					
0.01 mg/kg	7.3	7.2	7.6	7.7	8.4
0.1 mg/kg	7.4	7.1	7.7	7.8	8.4
1 mg/kg	7.4	7.3	7.7	7.8	8.3
10 mg/kg	7.4	7.3	7.7	7.8	8.3
100 mg/kg	7.4	7.3	7.7	7.8	8.3
Treatment (MIX)	Day 1	Day 8	Day 13	Day 20	Day 28
Temperature (°C)					
0.02 mg/kg	18.5	19	18.6	19.5	16.9
0.2 mg/kg	18.5	19.3	18.7	19.8	16.5
2 mg/kg	18.5	19.3	18.7	20	16.7
20 mg/kg	18.5	19.1	18.7	20.1	16.7
200 mg/kg	18.5	18.8	18.8	20.1	17
DO (%)					
0.02 mg/kg	97	93.4	95.4	98.1	113.3
0.2 mg/kg	98.6	98.2	93.7	98.6	115.5
2 mg/kg	98.7	95.5	93.9	93.3	110.8
20 mg/kg	99.2	96.8	92	91	113.2
200 mg/kg	99.5	96.4	94.9	91.6	111
pH					
0.02 mg/kg	7.5	7.3	7.7	7.8	8.4
0.2 mg/kg	7.5	7.4	7.7	7.9	8.4
2 mg/kg	7.5	7.4	7.7	7.9	8.4
20 mg/kg	7.5	7.4	7.7	7.9	8.3
200 mg/kg	7.5	7.4	7.7	7.9	8.2

Treatment (NC)	Day 1	Day 8	Day 13	Day 20	Day 28
Temperature (°C)	20.6	19.9	20.2	20.1	19.3
DO (%)	92.6	96.5	88.5	87.5	102.3
pH	6.7	6.8	7.4	7.7	8.2
Treatment (SC)	Day 1	Day 8	Day 13	Day 20	Day 28
Temperature (°C)	20.6	20.2	19.8	20	19.4
DO (%)	92.6	94.9	91.3	87.5	100.6
pH	6.9	6.8	7.5	7.8	8.3

6. Benoxacor Spike Recovery Data: Aqueous Samples Concentrations (uM)

DAY	Spiked DI water	Spiked M7 Medium	Spiked Sand	Spiked Sediment
0	79.461	80.505	27.064	10.765
1	79.707	81.579	42.964	17.978
2	79.983	80.536	52.910	25.253
3	79.676	82.255	56.839	29.642
4	78.63	80.167	57.606	32.528
8	78.295	81.549	64.359	41.675
12	70.867	83.697	66.170	42.749
16	80.751	76.115	68.718	42.136
20	79.707	83.605	68.779	39.557
24	86.245	80.628	70.375	37.807
28	84.741	81.948	67.797	32.221

Statistics Details by Endpoint

Emergence Ratio

Emergence ratio was calculated as the total number of adults (males and females pooled) emerged by day 28 out of twenty total larvae per microcosm. A Welch's t-test was used to test whether mean emergence ratios differed between negative control and solvent control groups, but the null hypothesis was not rejected for either of the two experimental blocks (Supplemental Data, Table 7), and the solvent control group was thus used as the control group in subsequent ANOVAs. Homogeneity of variance was tested with a Bartlett test. Inclusion of the high dose treatment for benoxacor data violated this assumption, but lower dose groups did have equal variances. Data for mono-chlorinated benoxacor, S-metolachlor, and the mixture all satisfied Bartlett's test (Supplemental Data, Table 8). Emergence ratios for all four experiments failed the Shapiro-Wilks test for normality (Supplemental Data, Table 9). ANOVA is considered robust to moderate deviations from the test assumptions. Thus, the tests were continued with raw emergence ratio values (after evaluating similar results using transformed survival data).

Emergence Rate

Male and female emergence rates were analyzed separately because males and females have different emergence rates (females typically emerge more slowly in culture and control conditions), as demonstrated by a Welch's t-test (Supplemental Data, Table 10). Replicates with no surviving adults were

disregarded for the analysis of emergence rate. Assumption test results can be found in Supplemental Data, Tables 11-14.

Adult Weight

Male and female dry weights were analyzed separately. A three-parameter Weibull model (lower limit set to 0) was applied to the data set of males treated with S-metolachlor (the only combination of chemical exposure and adult sex with a significant effect of treatment seen by ANOVA). Assumption test results can be found in Supplemental Data, Tables 15-18.

Cox proportional hazards modeling

Time-to-emergence curves were analyzed for males and females separately. Time to event analysis examines and models the time it takes for events to occur, or the survival time [27] (in this case the time to successful, healthy emergence). The number of successfully emerging, healthy males and females (every ~12 h) was fit to a Cox proportional-hazards model. Time (h) was the independent variable and proportion of successfully emerged (alive and actively mobile at the time of observation) individuals of each sex was the dependent variable. Larvae or pupae that were completely immobile on the sediment surface during the study, or those that did not emerge, were considered right-censored data. Adults that emerged but were already dead at observation were considered unhealthy. Thus these emerged adults were given a different code in the statistical software.

7. Welch's test results comparing emergence ratio in negative and solvent control groups

Block	t-value	df	p-value
1	0.49669	13.79	0.6272
2	-0.53846	4.9906	0.6134

8. Bartlett test results testing the homogeneity of variances of emergence ratios between each concentration of each chemical exposure

Chemical	K-squared	df	p-value
BN (high dose included)	17.084	5	0.004343
BN (high dose removed)	6.9089	4	0.1408
BNMCL	7.9215	5	0.1606
SM	1.7745	5	0.8794
MIX	6.439	5	0.2658

9. Shapiro-Wilks test for normality of data distribution

Chemical	W-statistic	p-value
BN	0.79719	3.611e-05
BNMCL	0.81882	9.394e-05
SM	0.92553	0.02944
MIX	0.75829	9.542e-06

10. Welch's t-test results, testing the difference in emergence rates between males and females within the solvent control group

t-statistic	Df	p-value
2.4916	21.906	0.0278

11. Bartlett test results: male emergence rates

Chemical	K-squared	df	p-value
BN	7.2371	5	0.2036
BNMCL	9.9366	5	0.07705
SM	1.6512	5	0.895
MIX	1.8398	5	0.8708

12. Bartlett test results: female emergence rates

Chemical	K-squared	df	p-value
BN	2.9036	5	0.7148
BNMCL	3.50979	5	0.6222
SM	2.5801	5	0.7644
MIX	5.7927	4	0.2152

13. Shapiro-Wilks test for normality: male emergence rates

Chemical	W-statistic	P-value
BN	0.90764	0.01298
BNMCL	0.92714	0.1534
SM	0.92944	0.1507
MIX	0.93357	0.0681

14. Shapiro-Wilks test for normality: female emergence rates

Chemical	W-statistic	P-value
BN	0.91455	0.01941
BNMCL	0.89796	0.03776
SM	0.94194	0.08501
MIX	0.95542	0.2702

15. Bartlett test results: male adult dry weights

Chemical	K-squared	df	p-value
BN	1.1726	5	0.9473
BNMCL	0.62908	5	0.9866
SM	2.3067	5	0.8053
MIX	14.532	5	0.01256

16. Bartlett test results: female adult dry weights

Chemical	K-squared	df	p-value
BN	0.24721	5	0.9985
BNMCL	0.81046	5	0.9764
SM	2.7907	5	0.7322
MIX	7.0168	4[a]	0.135

[a]Recieved an error that there must be at least two values in a group to run the Bartlett test (there was one average weight value for females in the high level). The high level was deleted to run the Bartlett test for the mixture.

17. Shapiro-Wilks test for normality: male adult dry weights

Chemical	W-statistic	P-value
BN	0.90825	0.01345
BNMCL	0.91327	0.0157
SM	0.94294	0.09076
MIX	0.94323	0.1218

18. Shapiro-Wilks test for normality: female adult dry weights

Chemical	W-statistic	P-value
BN	0.88104	0.002979
BNMCL	0.90354	0.007631
SM	0.95149	0.1589
MIX	0.92699	0.06178

19.
ANOVA source table: adult emergence ratio (survival)

Chemical	Parameter	Df	SS	Mean Sq	F value	Pr(>F)
BN	Treatment	5	0.770	0.154	3.996	0.00842**
	Block	1	0.053	0.053	1.393	0.24895
	Residuals	25	0.963	0.038		
BNMCL	Treatment	5	0.080	0.161	9.316	4.16e-05***
	Block	1	0.097	0.097	5.647	0.0255*
	Residuals	20	0.433	0.017		
SM	Treatment	5	0.319	0.063	6.078	0.00818***
	Block	1	0.177	0.177	16.884	0.000374***
	Residuals	25	0.262	0.010		
MIX	Treatment	5	1.745	0.349	22.637	2.29e-08***
	Block	1	0.112	0.112	7.294	0.0125*
	Residuals	24	0.370	0.015		

* $p < 0.05$.
** $p < 0.005$.
*** $p < 0.0005$.
BN = Benoxacor; BNMCL = Monochlorinated Benoxacor; SM = S-metolachlor; MIX = Mixture.

20. Three-parameter Wiebull model: adult emergence ratio (survival)

Chemical	Parameter	Estimate	Std. Error	T-value	p-value
BN	Slope	1.173	1.088	1.078	0.2897
	Y at 0	0.815	0.040	20.345	0.0000
	Inflection point	115.406	36.396	3.170	0.0036
BNMCL	Slope	0.476	0.111	4.274	2e-04
	Y at 0	0.830	0.035	23.512	0e+00
	Inflection point	483.739	17.337	27.901	0e+00
SM	Slope	2.434	2.948	0.825	0.4157
	Y at 0	0.810	0.024	33.473	0.0000
	Inflection point	141.014	61.295	2.300	0.0288
MIX	Slope	2.769	3.330	0.831	0.4127
	Y at 0	0.830	0.025	32.063	0.0000
	Inflection point	79.332	23.419	3.387	0.0021

Residual standard error and DF: Benoxacor (0.1897, 29 DF); Monochlorinated benoxacor (0.1669, 29 DF); S-metolachlor (0.1279, 29 DF); Mixture (0.1335, 28 DF).

21. Effects of benoxacor, monochlorinated benoxacor, S-metolachlor, and the mixture on emergence ratio of larval C. riparius[a]

	EC20 (95% CI)	EC50 (95% CI)	Slope ± SE	T-value (probability)
BN	32.16(-38.16-102.49)	84.45(31.124-137.79)	1.173±1.088	1.078(0.2897)
BNMCL	20.81(-10.525-52.154)	224.26(140.48-308.05)	0.476±0.111	4.274(2e-04)
SM	55.962(-25.42-137.35)	121.30(55.80-186.82)	2.434±2.948	0.825(0.5157)
MIX	46.157(-42.10-134.42)	69.49(5.75-133.24)	2.769±3.330	0.831(0.4127)

[a]The toxicity data are presented as EC20 and EC50 and their 95% confidence intervals (95% CI) in mg/kg, the concentrations at which 20% and 50% of tested midges were affected, respectively, in a 28-day bioassay.
BN = Benoxacor; BNMCL = Monochlorinated Benoxacor; SM = S-metolachlor; MIX = Mixture.

22. ANOVA source table: adult emergence rate

Chemical	Parameter	Df	Sum Sq	Mean Sq	F value	Pr(>F)
BN (Males)	Treatment	5	0.00016	3.314e-05	3.029	0.030340*
	Block	1	0.00016	1.601e-04	14.632	0.000867***
	Residuals	23	02.516e-04	1.094e-05		
BN (Females)	Treatment	5	0.00016	3.29e-05	3.77	0.012224*
	Block	1	0.00016	1.601e-04	18.51	0.000265**
	Residuals	23	0.00019	8.650e-06		
MIX (Males)	Treatment	5	0.00031	6.329e-05	18.35	3.41e-07***
	Block	1	0.00018	1.827e-04	52.97	2.74e-07***
	Residuals	22	0.00007	3.450e-06		
MIX (Females)	Treatment	5	0.00011	2.392e-05	3.461	0.019457*
	Block	1	0.00011	1.180e-04	17.071	0.000474***
	Residuals	21	0.00014	6.910e-06		

* $p < 0.05$.
** $p < 0.005$.
*** $p < 0.0005$.
BN = Benoxacor; BNMCL = Monochlorinated Benoxacor; SM = S-metolachlor; MIX = Mixture.

23. Three-parameter Weibull model and subsequent ED20 and ED50 values: adult emergence rate

Group	Parameter	Estimate	Std. Error	T-value	p-value
BN Males	Slope	5.2237e-01	3.78e-01	1.3814e+00	0.1785
	Y at 0	6.3299e-02	9.2099e-04	6.8729e+01	0.0000
	Inflection point	4.1668e+03	1.3188e+04	3.1595e-01	0.7545
BN Females	Slope	4.368e-01	NA	NA	NA
	Y at 0	6.0207e-02	8.1390e-04	7.39743+01	0
	Inflection point	9.288e+03	NA	NA	NA
MIX Males	Slope	7.3903e-01	4.4648e-01	1.6552e+00	0.1099
	Y at 0	6.2801e-02	7.7656e-04	8.0871e+01	0.0000
	Inflection point	8.3435e+02	1.2422e+03	6.7169e-01	0.5077
MIX Females	Slope	2.0952e-01	5.2371e-02	4.0007e+00	0.0005
	Y at 0	6.0162e-02	9.3849e-04	6.4105e+01	0.0000
	Inflection point	8.6117e+07	2.0751e+08	4.1501e-01	0.6817

Residual standard error and DF: Benoxacor males (0.0040, 27 DF); Benoxacor females (0.003735, 27 DF); Mixture males (0.003409, 26 DF); Mixture females (0.0038, 25 DF).

24. ED10 and ED20 estimates for male and female emergence rates when exposed to BN and the MIX: estimated from the three-parameter Weibull curve fit to the data.

Group	ED	Estimate	Std. Error	Lower	Upper
BN Males	1:10	56.086	39.197	-24.340	136.51
	1:20	235.915	289.348	-357.778	829.61
BN Females	1:10	NaN	NaN	NaN	NaN
	1:20	NaN	NaN	NaN	NaN
MIX Males	1:10	39.7105	19.8965	-1.1873	80.608
	1:20	109.6221	43.0497	21.1321	198.112
MIX Females	1:10	1864.2	4739.1	-7896.3	11625
	1:20	66986.1	144321.4	-230249	364222

25. ANOVA source table: adult male dry body mass (g)

Group	Parameter	Df	SS	Mean Sq	F value	Pr(>F)
SM males	Treatment	5	0.098	0.0196	6.244	0.000690***
	Block	1	0.066	0.0661	21.051	0.000108***
	Residuals	25	0.078	0.0031		

* p < 0.05.
** p < 0.005.
*** p < 0.0005.
BN = Benoxacor; BNMCL = Monochlorinated Benoxacor; SM = S-metolachlor; MIX = Mixture.

26. Three-parameter Wiebull model and subsequent ED20 and ED50 values: males exposed to S-metolachlor, dry body mass

Parameter	Estimate	Std. Error	t-value	p-value
Slope	0.407	0.255	1.594	0.1217
Y at 0	0.390	0.021	18.566	0.0000
Inflection point	416.814	476.326	0.875	0.3887
ED	Estimate	Std. Error	Lower	Upper
10	1.674	4.348	-7.219	10.568
20	10.540	15.882	-21.942	43.023

ANOVA Residual standard error: 0.07216688 (29 degrees of freedom).
* p < 0.05.
** p < 0.005.
*** p < 0.0005.
BN = Benoxacor; BNMCL = Monochlorinated Benoxacor; SM = S-metolachlor; MIX = Mixture.

27a. Abnormal larval behavior observed: Block 1

Day							
BN	**11**	**12**	**14**	**16**	**17**	**19**	**20**
Treatment							
(mg/kg)						100	100
Replicate						B	B
Behavior						L	L
# Individuals						1	1
BNMCL							
Treatment							
(mg/kg)				100	100		
Replicate				B	B		
Behavior				L	L		
# Individuals				1	1		
SM							
Treatment							
(mg/kg)			100				
Replicate			A				
Behavior			L				
# Individuals			1				
MIX							
Treatment							
(mg/kg)	200	200					
Replicate	B	B					
Behavior	L	L					
# Individuals	1	1					

BN = Benoxacor; BNMCL = Monochlorinated Benoxacor; SM = S-metolachlor; MIX = Mixture. L = Lethargic;

27b. Abnormal larval behavior observed: Block 2

Day	11	12	13	14	15	16	17	18	19	20
BN										
Treatment (mg/kg)	100	100	100	100	100	100	100	100		100
Replicate	B	B	B	B	B	B	B	B		B
Behavior	L	L	L/C	C, P	C	L	C	L		L
# Individuals	6	6	4	5, 2	12	5	6	2		1
Treatment		100	100	100	100	100	100	100	100	100
Replicate		A	A	A	A	A	A	A	A	A
Behavior		L	L	P	L	L	L	L	L	L
# Individuals		1	2	2	2	1	3	2	2	2
BNMCL										
Treatment (mg/kg)			100			100	100			
Replicate			B			B	B			
Behavior			L			L	L			
# Individuals			1			1	1			
Treatment				100						
Replicate				A						
Behavior				P						
# Individuals				2						
SM										
Treatment (mg/kg)				100		100				
Replicate				A		A				
Behavior				L		L				
# Individuals				2		1				
Treatment				100		100	100	100		
Replicate				B		B	B	B		
Behavior				L		L	L	L		
# Individuals				1		1	1	1		
MIX										
Treatment (mg/kg)	200	200	200		200					
Replicate	B	B	B		B					
Behavior	L	L, P	L		L					
# Individuals	4	3, 2	2		2					
Treatment		200	200	200	200					
Replicate		A	A	A	A					
Behavior		P	L, P	L	L					
# Individuals		3	3, 2	2	4					

BN = Benoxacor; BNMCL = Monochlorinated Benoxacor; SM = S-metolachlor; MIX = Mixture. L = Lethargic; P = Posterior of organism protruding still or waiving outside of the sediment burrow; C = Convulsing/writing on the sediment surface

Table 28. Emergence ratios of larval chironomids exposed as larvae to solvent control, benoxacor, mono-chlorinated benoxacor, S-metolachlor, and the mixture[a]

Chemical	Median nominal sediment concentration (mg/kg)	Adult Emergence Ratio (%)
SC	0	0.808±0.116
BN	0.042	0.888±0.075
	0.1135	0.788±0.170
	0.9685	0.788±0.246
	11.07	0.775±0.065
	109.64	0.350±0.436*
BNMCL	0.0435	0.863±0.048
	0.142	0.800±0.129
	0.983	0.813±0.160
	6.82	0.913±0.111
	116.1	0.363±0.266***
SM	0.0155	0.838±0.111
	0.101	0.763±0.144
	1.01	0.775±0.126
	14.89	0.875±0.104
	111.74	0.525±0.194*
MIX	0.014	0.817±0.029[b]
	0.101	0.875±0.119
	1.301	0.838±0.131
	10.33	0.850±0.178
	89.6	0.125±0.218***

[a]Data are expressed as means ± standard deviation, N = 4 unless noted.
[b]N=3, a replicate was mistakenly not initiated.
* $p < 0.05$.
*** $p < 0.0005$.
BN = Benoxacor; BNMCL = Monochlorinated Benoxacor; SM = S-metolachlor; MIX = Mixture.

Table 29. Emergence rates of male and female adult chironomids exposed as larvae to solvent control, benoxacor, monochlorinated benoxacor, S-metolachlor, and the mixture[a]

Chemical	Median nominal sediment concentration (mg/kg)	Emergence Rate Males (%)	Emergence Rate Females (%)
SC	0	0.063±0.003	0.059±0.003
BN	0.042	0.062±0.002	0.060±0.003
	0.1135	0.065±0.004	0.060±0.003
	0.9685	0.063±0.006	0.060±0.005
	11.07	0.061±0.003	0.059±0.003
	109.64	0.055±0.010[b]*	0.050±0.007[b]*
BNMCL	0.0435	0.062±0.003	0.058±0.003
	0.142	0.063±0.002	0.060±0.002
	0.983	0.062±0.006	0.060±0.003
	6.82	0.059±0.001	0.057±0.001
	116.1	0.058±0.000[c]	0.058±0.001
SM	0.0155	0.063±0.004	0.059±0.003
	0.101	0.062±0.004	0.059±0.003
	1.01	0.063±0.004	0.061±0.005
	14.89	0.064±0.005	0.060±0.005
	111.74	0.059±0.003	0.058±0.003
MIX	0.014	0.059±0.001[d]	0.056±0.0005[d]
	0.101	0063±0.004	0.061±0.003
	1.301	0.064±0.003	0.061±0.004
	10.33	0.061±0.004	0.059±0.004
	89.6	0.051±0.003[b]***	---[e]*

[a]Data are expressed as means ± standard deviation, N = 4 unless noted.
[b]N=2 (2 replicates had 0% survival and emergence rate was not calculated/included).
[c]N=3, males only emerged out of three replicates.
[d]N=3, a replicate was mistakenly not initiated.
[e]N=1, females emerged out of one replicate only, avg. emergence rate = 0.051
* $p < 0.05$.
*** $p < 0.0005$.
BN = Benoxacor; BNMCL = Monochlorinated Benoxacor; SM = S-metolachlor; MIX = Mixture.

Table 30. Dry body mass of male and female adult chironomids exposed as larvae to solvent control, benoxacor, monochlorinated benoxacor, S-metolachlor, and the mixture[a]

Chemical	Median nominal sediment concentration (mg/kg)	Average Body Male Body Mass (g)	Average Female Body Mass (g)
SC	0	0.398±0.084	0.750±0.167
BN	0.042	0.383±0.099	0.698±0.164
	0.1135	0.403±0.083	0.768±0.146
	0.9685	0.425±0.112	0.781±0.189
	11.07	0.393±0.089	0.727±0.187
	109.64	0.448±0.040[b]	0.797±0.154[b]
BNMCL	0.0435	0.363±0.059	0.685±0.178
	0.142	0.390±0.062	0.722±0.139
	0.983	0.407±0.078	0.780±0.215
	6.82	0.367±0.076	0.716±0.175
	116.1	0.312±0.080[c]	0.664±0.139
SM	0.0155	0.380±0.037	0.736±0.097
	0.101	0.358±0.067	0.768±0.129
	1.01	0.337±0.066	0.710±0.091
	14.89	0.337±0.066	0.688±0.095
	111.74	0.222±0.088**	0.643±0.115
MIX	0.014	0.368±0.033[c]	0.714±0.021[c]
	0.101	0.376±0.059	0.751±0.124
	1.301	0.384±0.007	0.814±0.077
	10.33	0.341±0.025	0.715±0.153
	89.6	0.306±0.117[b]	---[d]

[a]Data are expressed as means ± standard deviation; N = 4 unless noted.
[b]N=2
[c]N=3
[d]N=1, average emergence rate = 0.556
**$p < 0.005$.
BN = Benoxacor; BNMCL = Monochlorinated Benoxacor; SM = S-metolachlor; MIX = Mixture.