Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/6/43/eabc1299/DC1

Supplementary Materials for

Common insecticide disrupts aquatic communities: A mesocosm-to-field ecological risk assessment of fipronil and its degradates in U.S. streams

Janet L. Miller, Travis S. Schmidt*, Peter C. Van Metre, Barbara J. Mahler, Mark W. Sandstrom, Lisa H. Nowell, Daren M. Carlisle, Patrick W. Moran

*Corresponding author. Email: tschmidt@usgs.gov

Published 23 October 2020, *Sci. Adv.* **6**, eabc1299 (2020)
DOI: 10.1126/sciadv.abc1299

This PDF file includes:

Mesocosm Experimental Design

The 30-day mesocosm experiment was run at the USGS Aquatic Experimental Laboratory (AXL), Fort Collins, Colorado, from October 18 to November 17, 2017 (1 day for acclimation plus a 30-day experiment). Methods have been previously described (*29, 31*). The mesocosm setup consisted of 36 recirculating stream mesocosms contained within four Living Streams® (a recirculating water tank). Each Living Stream® is equipped with a chiller to maintain water temperature and illuminated by four Verilux® bulbs (27 µmol m^{-2} s^{-1}) on a 16:8 L:D cycle. The stream mesocosms were stainless steel, suitable for the hydrophobicity of fipronil (log k_{ow}=4.0) and for use with organic cleaning solvents (Figure S1); the recirculating pump (Danner Supreme® model 3) and connector (Polyvinyl chloride, PVC) to the stainless-steel stream mesocosm were made of plastic.

Water for the mesocosm experiment was collected from the Cache La Poudre River (headwaters include Rocky Mountain National Park, National Forest, and the Continental Divide) and stored in four 2,643-L (600-gal) polyethylene Ace Roto-Mold® tanks at the AXL. Previous assessment of sediment and water samples collected from this site returned no pesticide detections (*29*). The water tanks were outfitted with pumps and plumbing such that all water was mixed among tanks and then distributed to four head tanks located above the Living Streams®. Water was delivered to each stream mesocosm via two peristaltic pumps—one for clean river water stored in the head tanks and one for treatment water (water spiked with a fipronil compound) stored in 20-L stainless-steel containers with lids—each at 2 mL/min for a total replacement rate of 4 mL/min or a little more than one complete water turnover per day. Flow rates were measured and recorded daily and adjusted if required. Natural benthic

communities transplanted from the Cache La Poudre River into the mesocosms were exposed exclusively to clean river water for the first day to acclimate the invertebrates to experimental conditions. The pumps for the treatment water were started on day 1 of the experiment.

The mesocosm experimental design consisted of 30 treatment streams and 6 control streams. Treatment streams received treated water, each consisting of an un-replicated constant concentration of a fipronil compound: fipronil (Sigma-Aldrich, CAS 120068-37-3), amide (Sigma-Aldrich, CAS 205650-69-7), desulfinyl (U.S. Environmental Protection Agency (EPA) Pesticide Repository, CAS 205650-65-3), sulfone (Sigma-Aldrich, CAS 120068-37-2), and sulfide (Sigma-Aldrich, CAS 120067-83-6); all purities were ≥ 97.8%. Nominal exposure concentrations ranged from 0.002 to 15.625 µg/L (fipronil and amide) and from 0.001 to 3.125 µg/L (desulfinyl, sulfone, and sulfide), chosen based on published response values (*7, 15, 16, 18, 21, 23, 25, 32, 33*). Concentrated stock solutions were prepared by dissolving a fipronil compound in methanol (Fisher certified ACS grade) and diluting with deionized water to the desired volume in a volumetric flask. Each stock solution was serially diluted, resulting in six individual stock solutions per compound, stored in amber glass in the dark at 4 ˚C, and used to dose treatment water throughout the mesocosm experiment. Appropriate volume aliquots of the solutions were used to spike 20-L polyethylene carboys of river water to achieve twice the targeted exposure concentration. Because of the different amounts of methanol in a dose, methanol was added to all stream mesocosms and to three of the control mesocosms as needed to ensure the same methanol concentration (0.05 mL/L) among stream mesocosms. Carboys used to fill the lidded stainless-steel dose containers above the streams were covered

to reduce photolysis. The remaining three control stream mesocosms received 4 mL/min clean river water, no methanol, and were otherwise treated as all other streams.

Temperature, pH, conductivity, and fipronil and fipronil degradates were measured in the stream mesocosms on days 8, 16, and 26. To track degradation of the parent compound fipronil over the duration of the mesocosm experiment the fipronil (parent)-treatment stream mesocosms were sampled on 3 additional days (days 5, 12, and 21, [n=6]) for temperature, pH, conductivity, fipronil, and the fipronil degradates. Temperature, pH, and conductivity were measured with a Hach HQ40d field meter. Samples for pesticide analysis were collected by filtering 10 mL of stream water through a Whatman 0.7-µm GF/F syringe filter equipped with a large-bore needle into a 20-mL amber glass vial. Samples were immediately frozen and sent to the USGS National Water Quality Laboratory (NWQL), Lakewood, Colorado, where they were analyzed for pesticides on February 2 and 9, 2018. Fipronil and the four degradates were measured in water samples by direct aqueous-injection (DAI) liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a modification of a previously published method (*34*). The modification was the use of a more sensitive LC-MS/MS instrument (Agilent 6495) that allows injection of less sample (20 µL) to minimize bias from sample matrix. Instrument detection levels (IDLs) were estimated as the lowest calibration standard that met qualitative identification criteria; IDLs were 0.005 µg/L for fipronil amide and 0.001 µg/L for the other four fipronil compounds. A complete description of the method used to measure fipronil compounds, including quality control and assurance procedures (e.g., sample recoveries, spikes, third-party checks, and blanks), is provided below.

Enumeration and identification of adult and larval invertebrates, major data collection endpoints, were done at the end of the 30-day mesocosm experiment. Emergent adult insects were collected from nets daily and frozen in clean 15-mL Falcon® centrifuge tubes. At the end of the experiment (day 30), the rocks, rock trays, and the interior of each stream mesocosm were scrubbed to dislodge any invertebrates, and contents were sieved at 500 and 250 µm successively, preserving the contents of each sieve separately in 80% ethanol. Taxonomic identification of larval and adult invertebrates was completed by Timberline Aquatics (Fort Collins, Colorado) to the lowest taxonomic level possible, typically species. Chlorophyll *a* was measured in every stream mesocosm on days 9, 19, and 29 in triplicate using a BenthoTorch (bbe Moldaenke GmbH, Germany) portable fluorometer that provides real-time qualitative and quantitative in-situ data (*64*). All chemical and biological data generated by the mesocosm experiment are in the companion data release (*35*).

Field invertebrate sampling methods

Invertebrate communities were sampled at the end of each regional study (spring/summer); typically, coincident with the last water-quality sampling event. The water-quality sampling was timed to coincide with low-flow conditions following the growing season and high pesticide use and with the time when stream invertebrate communities are mature and predominantly in the larval life stage. Invertebrate community sampling was completed at 437 of the 444 sites. In the Midwest and Coastal California regional studies, invertebrates were sampled at 11 equally spaced transects at one of the assigned channel locations (either left, center, or right) using a D-frame net (500 µm) (*65*) and preserved in 95% ethanol, with larger

predaceous invertebrates preserved immediately to reduce consumption of or damage to other organisms. In the Southwest, Pacific Northwest, and Northeast regional studies, invertebrate sampling targeted rich habitats such as riffles or, in low-gradient streams, woody snags; samples were collected using a modified Surber sampler with 500-µm mesh net from a total sampled area of 1.25 m^2 and composited. These invertebrate samples were sieved through a 500-µm sieve, large organic and inorganic debris was removed, and the sample was preserved with 10% buffered formalin (*36-40*). All invertebrates were identified and enumerated generally at either genus or species level at the NWQL in Denver, Colorado. The two macroinvertebrate sampling protocols used here were comparable in the number of composite samples, total sampled area, use of quantitative collection techniques (either Surber sampler or D-frame nets with 500-µm mesh openings), stream-reach length (typically 150 to 300 m), and common laboratory procedures. Assessment of ecological condition based on Ephemeroptera + Plecoptera + Tricoptera (EPT) richness was previously reported (*66*) to be consistent for transect-based versus targeted sampling such as were used in the present study.

Data analysis

Effect concentrations at which there was a 20% or 50% reduction in larval invertebrates relative to controls (i.e., EC_{20}, EC_{50}) were calculated for each of the five fipronil compounds used in the mesocosm experiment. Exposure concentrations were characterized by time-weighted averages of measured concentrations, whereby each observation was weighted by the number of days between the start of the mesocosm experiment and the next observation, between observations, or time of last observation to the end of the experiment. If a compound was not detected in a stream mesocosm and the nominal (treatment target concentration)

concentration was >0, an estimated value of 0.5 times the IDL was substituted into the analysis (substitutions were made for 11 of 108 target compound measurements); this estimated value was at or below the lowest nominal concentration for all compounds except fipronil amide. The data (x=time-weighted fipronil concentration, y=larval abundance or other metric) were fit in R (*43*) extension package "drc" using a three-parameter logistic regression (*44*). Curves were fit to all (larvae) individual species with adequate abundance and to additional metrics of interest (e.g., taxa richness, total mayfly abundance, total abundance) to further understand community effects. All analyses of larval metrics used the total larvae collected for a given stream mesocosm (500-μm + 250-μm sieves). Model fit was assessed using the Nash-Sutcliff Coefficient (NSC) (*45*) where a poor model fit can receive infinitely negative values and perfect fit receives a value of 1. The NSC is a more effective measure of non-linear model fit than Root Mean Squared Error or (R^2) (*67*).

To explore the effects of fipronil compounds on insect emergence in the experiment, data were evaluated in two ways. First, the cumulative daily emergence (count of all individuals) of insects from each stream mesocosm was tabulated. The cumulative daily emergence was then normalized to controls by subtracting the mean emergence in the control stream mesocosms from the emergence in each treatment stream mesocosm. These values were plotted against time to understand how emergence in the treatment stream mesocosms deviated from the control stream mesocosms over the 30-day experiment. Secondly, the percent total emergence for each stream mesocosm, defined as the ratio of the total number of emergers from a given stream mesocosm to the mean number of larvae and adults in the controls, also was calculated. The percent total emergence (y) as a function of concentration

(x=time weighted average compound concentration) was fit to a three-parameter logistic regression for each fipronil compound to estimate effect concentrations on emergence, as was done for estimating effect concentrations for larval mortality (R extension package "drc"). This allowed comparison of effect concentrations of two different endpoints, larval mortality and adult emergence. All emergent insects collected were from two subfamilies of the family *Chironomidae* and therefore were combined for analysis.

Changes to community structure, such as taxa loss, can result in changes to community function. Indirect effects, known as a trophic cascade, can be explored through structural equation modeling. To test for a trophic cascade, a simple network of cause−effect relationships were evaluated using a path-analytic approach (R package "piecewiseSEM") (*46*). For the mesocosm experiment, the presence of fipronil, desulfinyl, sulfide, and sulfone in water was hypothesized to reduce the biomass of scrapers, indirectly causing an increase in the biomass of chlorophyll *a* (*47*). Compound concentrations were the predictor variables, and scraper and chlorophyll *a* biomass were response variables. To calculate biomass, length measurements of the individual larvae were converted to mass using a length/mass regression based on known relations between length and mass for each taxon (*68*) and then assigned to their appropriate functional feeding group (e.g., collector -gatherer, collector-filterer, scraper, predator, shredder) to determine total biomass for each functional feeding group (FFG). Amide was not tested because the experiment resulted in few dose-response relations between amide and invertebrates. Exposure concentrations and scraper biomass were $\log_{10}(X+1)$ transformed, and chlorophyll *a* was $\log_{10}$ transformed to improve the distribution of residuals, which are

assumed to achieve multivariate normality. The Fisher's C statistic was used to assess model fit such that p-values < 0.05 indicate good model fit (*46*).

To develop a risk-based threshold protective of ecological communities, chronic species sensitivity distributions (SSD) and hazard concentrations protective of 95% of the affected species (HC_5, hazard concentration for 5% of affected species) were derived for each compound. Three SSD datasets were generated: (1) a mesocosm-only dataset, (2) a dataset inclusive of all the mesocosm data combined with that collected from a query of the U.S. Environmental Protection Agency ECOTOX database (https://cfpub.epa.gov/ecotox/, accessed 3/14/2019), and (3) a dataset inclusive of all the mesocosm data and the ECOTOX data where the ECOTOX data (acute exposures) were divided by the acute-to-chronic ratio for *Daphnia magna* (19.39) as a means to account for differences in exposure duration and to approximate chronic EC_{50} values (*12*). Data collected from the ECOTOX database were evaluated on the following criteria: inclusive of exposures $\geq$ 4 days, tests conducted in the laboratory using fresh water, included at least one measurement of exposure concentrations, used active ingredient to develop exposures, and reported mortality as an EC_{50} or lethal concentration that elicits a 50% response (LC_{50}). No data from the ECOTOX database had an exposure longer than 4 days. If the more than one result was reported for a taxon, the lowest EC_{50} was used for SSD development. Our purposes in generating multiple SSD models were (1) to develop HC_5 values for comparison with field data (mesocosm-only SSD) and, (2) evaluate the robustness of mesocosm data relative to data resources more broadly accepted by regulators for inclusion into aquatic life benchmarks and criterion development and thus the utility of mesocosm studies for use in regulatory processes.

SSDs were developed for each dataset using the R package "ssdtools"(*48*). Maximum likelihood methods were used to fit multiple data distributions (log-normal, log-gumbel, gamma, and weibull) to each SSD dataset, and the best-fit distribution was selected using Akaike Information Criterion (AIC) (*69*). An HC_5 was estimated from the best-fit cumulative distribution of each SSD dataset, and confidence intervals (CI) were estimated using a bootstrapping (n=10,000) procedure. 49 taxa responses developed from this study (all taxa identified to genus or species) were combined with 32 taxa responses compiled from 6 published studies found in the ECOTOX database, combining for a total of 81 taxa responses for use in SSD development. No SSD was developed for amide, as no data were found in the ECOTOX database for amide, and only one EC_{50} response was derived from the current study. While only one EC_{50} for desulfinyl was found in the ECOTOX database, 12 EC_{50} values were generated by the current study, therefore SSDs were developed for desulfinyl.

The fipronil compound-specific HC_5 values derived from the mesocosm-only SSD dataset were combined with field data to estimate exposure and potential toxicity to fipronil compounds in the 444 streams from the five U.S. regions assessed. Each detected concentration (non-detections treated as zeros) of a fipronil compound during the final 4-week sampling window was divided by its respective HC_5, and the compound ratios for each sample were summed to obtain the total toxic units for fipronil(s) ($\Sigma TU_{Fipronils}$) for that sample, where $\Sigma TU_{Fipronils} > 1$ indicates toxicity. The rationale for replacing non-detections with zero was that a non-detection could mean the pesticide did not occur in the stream and, from a toxicological perspective, is a conservative approach to avoid inflating toxic unit values because of the limits of analytical methods. This contrasts with the mesocosm experiment, for which non-detections

were replaced with one-half the IDL when known pesticide was added to the treatment; here, occurrence of the pesticide is a given.

The degree to which the SSD derived from the mesocosm data reflects the broader ecological community sensitivity to fipronil(s) was evaluated using a method similar to that described above. A Hazard Concentration for 50% of affected species (HC_{50}) was calculated for each compound and compared to the EC_{50} value for taxa richness developed from the mesocosm experiment. This comparison allows for an evaluation of the agreement between the SSD approach, which includes only those taxa responses with dose-response relationships, and the EC_{50} approach using the metric taxa richness, which includes all unique taxa observed in the mesocosms, including those that did not have a dose-response relationship.

A Species at Risk for pesticides ($SPEAR_{pesticides}$) metric was calculated to investigate the relation between the health of the invertebrate community and $\Sigma TU_{Fipronils}$ at the 437 stream sites where invertebrates were collected. The $SPEAR_{pesticides}$ is a metric that converts invertebrate composition into physiological and ecological traits that indicate community susceptibility to pesticides and is insensitive to natural covariates (*49, 70*), although performance of the $SPEAR_{pesticides}$ metric can be affected by severe habitat degradation (*51*). The field-collected abundance data for each taxonomic unit were harmonized with the taxa key values associated with the ASTERICS software for assessing the ecological quality of rivers (https://www.gewaesser-bewertung-berechnung.de/index.php/home.html). The data then were imported into the Indicate (http://www.systemecology.eu/indicate/) software (version 18.05) where, using a European trait database and a database of physiological sensitivity to

pesticides, the data at each site were converted into a $SPEAR_{pesticides}$ metric. $SPEAR_{pesticides}$ is the relative taxa abundance at each site calculated as follows:

$$SPEAR_{pesticides} = \frac{\sum_{i=1}^{n} \log(4x_i+1)*y}{\sum_{i=1}^{n} \log(4x_i+1)}$$

where *n* is the number of taxa, χ_i is the abundance of the taxon *i* and *y* is 1 if taxon *i* is classified as ''at risk'', otherwise 0 (*49, 70*). Thus, $SPEAR_{pesticides}$ can range from 0, indicating no taxa sensitive to pesticides to a larger number equal to the relative abundance of taxa sensitive, or "at risk", to exposure to pesticide. $SPEAR_{pesticides}$ is then normalized by a predetermined average reference condition regarding "toxic pressure"(*70*). $SPEAR_{pesticides}$ ranges from 0 to >1 if local conditions have more species at risk than that in the average reference condition. General additive models (GAMs) ("mgcv" package in R (*52*)) were used to explore associations between the $SPEAR_{pesticides}$ metric and $\Sigma TU_{Fipronils}$ ($\log_{10}(X+1)$ transformed) for each of the five regional studies. The GAMs relate a univariate response variable (invertebrate metric) with predictors that are dependent on unknown smoother functions and were used to avoid assumption of the shape of the modeled relations.

Method for Determination of Fipronil Compounds in Mesocosm Water Samples

Fipronil and fipronil degradate compounds (fipronils) were determined in water samples by direct aqueous-injection (DAI) liquid chromatography-tandem mass spectrometry (LC-MS/MS). The method was a modification of a previously published method for 227 pesticide compounds (*34*). The modification was the use of a more sensitive LC-MS/MS instrument (Agilent 6495) that allowed injection of less sample (20 µL) to minimize bias from sample matrix.

Water samples were analyzed with an Agilent 1290 Infinity LC system consisting of a binary pump, autosampler, sample tray cooler, and heated column compartment, coupled to an Agilent 6495

triple quadrupole LC-MS/MS system equipped with an Agilent Jet Stream electrospray ionization source. The analytical column used for compound separation was a Zorbax C18 column (50 mm x 2.1 mm i.d., 3.5 µm particle size), maintained at 50°C. The mobile phase consisted of 3.5 mM (0.02% v/v) acetic acid in water (phase A) and acetonitrile (phase B). The gradient started with 90 percent phase A, changed to 60 percent at 4 min, 40 percent at 9 min, 5 percent at 13 min. The flow rate was 0.6 mL/min, and the injection volume was 20 µL.

Tandem mass spectrometry was carried out using negative electrospray ionization and multiple reaction monitoring (MRM). The precursor and product ions and optimum collision energy were selected from analysis of each individual compound. The retention times and precursor and product ions for each compound are available in Table 1 of the companion data release (*35*). The precursor ion obtained was the molecular ion [M-H]- for all compounds. Isotopically labeled standards, diuron-d_6 and ibuprofen-$^{13}C_3$, were added to every sample and used for quantitation. Qualitative identification criteria were that both MRM peaks overlapped and had retention times within 0.1 min of calibration standards, and MRM ion ratios from samples were within ±30% (relative) of calibration standards.

Results for Determination of Fipronil Compounds in Mesocosm Water Samples

Samples were analyzed in a sequence of instrument blanks, calibration standards, laboratory quality-control (QC) samples, and environmental samples called an analytical batch. The laboratory QC samples include continuing calibration verification (CCV) standards, instrument detection level (IDL) standards, and third-party check (TPC) standards. The laboratory reagent blanks and laboratory reagent spikes were prepared in sample containers, similar to the environmental samples. Low concentration standards were used as instrument detection level (IDL) standards to check LC-MS/MS response during and at the end of the analytical batch. A typical analytical batch consists of about 109 analytical vials

with 75 environmental samples, 2 laboratory QC samples, and 32 instrument QC samples (blanks, calibration standards, CCVs).

The stream mesocosm samples were analyzed in two batches. Data from each analytical batch were analyzed using the MassHunter quantitative analysis software (*71*). Calibration was performed using the peak areas and the internal standard technique. A series of 12 calibration standards, ranging from 1 to 10,000 ng/L, were analyzed at the start of each batch. Calibration curves were generated using the MassHunter software using quadratic curve fit, ignore origin, and 1/x weighting settings. The calibration curves used for the stream mesocosm samples met acceptance criteria of the fit of quadratic curve (R^2 greater than 0.99), and bias of each calibration standard in the curve relative to the nominal concentration was less than ±30 percent, except at the lowest level where bias was ±50 percent (*34*).

Isotopically labeled standards linuron-d_6 and ibuprofen-$^{13}C_3$ were used as internal standards for quantitation and were added to all laboratory QC samples and stream mesocosm samples. Linuron-d_6 was used for quantitation of the fipronils, and ibuprofen-$^{13}C_3$ was used for quantitation of linuron-d_6. The recoveries of the internal standard linuron-d_6 are shown in Table 2 of the companion data release (*35*). Median recoveries of linuron-d_6 were 103 percent (92 to 130 percent) in laboratory QC samples and 100 percent (79 to 139 percent) in mesocosm samples and met the method acceptance criteria (70-130 percent) (*34*).

Expected concentrations and recoveries of fortified compounds in the laboratory QC samples are shown in Table 3 of the companion data release (*35*). Median recoveries of all QC sample types for all compounds were within acceptance criteria used for the original method (70 to 130 percent) except for desulfinylfipronil in TPC samples (62 percent) and fipronil sulfone in 25 ng/L IDL standards (65 percent). Two CCV standards had relatively high recoveries (175 to 183 percent) for all compounds. The IDL standards at 5 ng/L were within acceptance criteria for all compounds, indicating that the quantitation method was accurate at least as low as this concentration.

The laboratory instrument blank and reagent blank samples analyzed with the stream mesocosm samples are summarized in Table 4 of the companion data release (*35*). There were no detections of desulfinylfipronil, fipronil amide, or fipronil sulfide in any of the blank samples. There was one detection of fipronil in an instrument blank (0.24 ng/L), and three detections of fipronil sulfone (maximum 0.23 ng/L). These low concentrations determined in the blank samples were less than 3 times the lowest concentration measured in the mesocosm stream samples, so no sample results were censored, although some associated samples did have a qualifier code added (*34*). The IDLs were estimated as the lowest calibration standard that met qualitative identification criteria, which were 5 ng/L for fipronil amide and 1 ng/L for all other fipronil compounds. Samples with no MRM response or that failed qualitative identification criteria were reported as <IDL.

Figure S1. Example of an experimental stream. Each stream mesocosm is constructed from a 15.1-L stainless-steel pot, outfitted with a magnetic-drive pump (Danner Supreme® model 3). The stream contains a 12.7-cm stainless-steel drainpipe covered with 1-mm metal mesh and a 21-cm perforated stainless-steel recirculation pipe surrounded with 1-mm wire mesh. The pump is connected on one end to recirculation pipe and on the other end to a stainless-steel elbow joint located above the water level to provide water flow and create an aerated ripple environment. Each stream contains four rock trays colonized with natural communities. Photo credit: Janet Miller, U.S. Geological Survey.

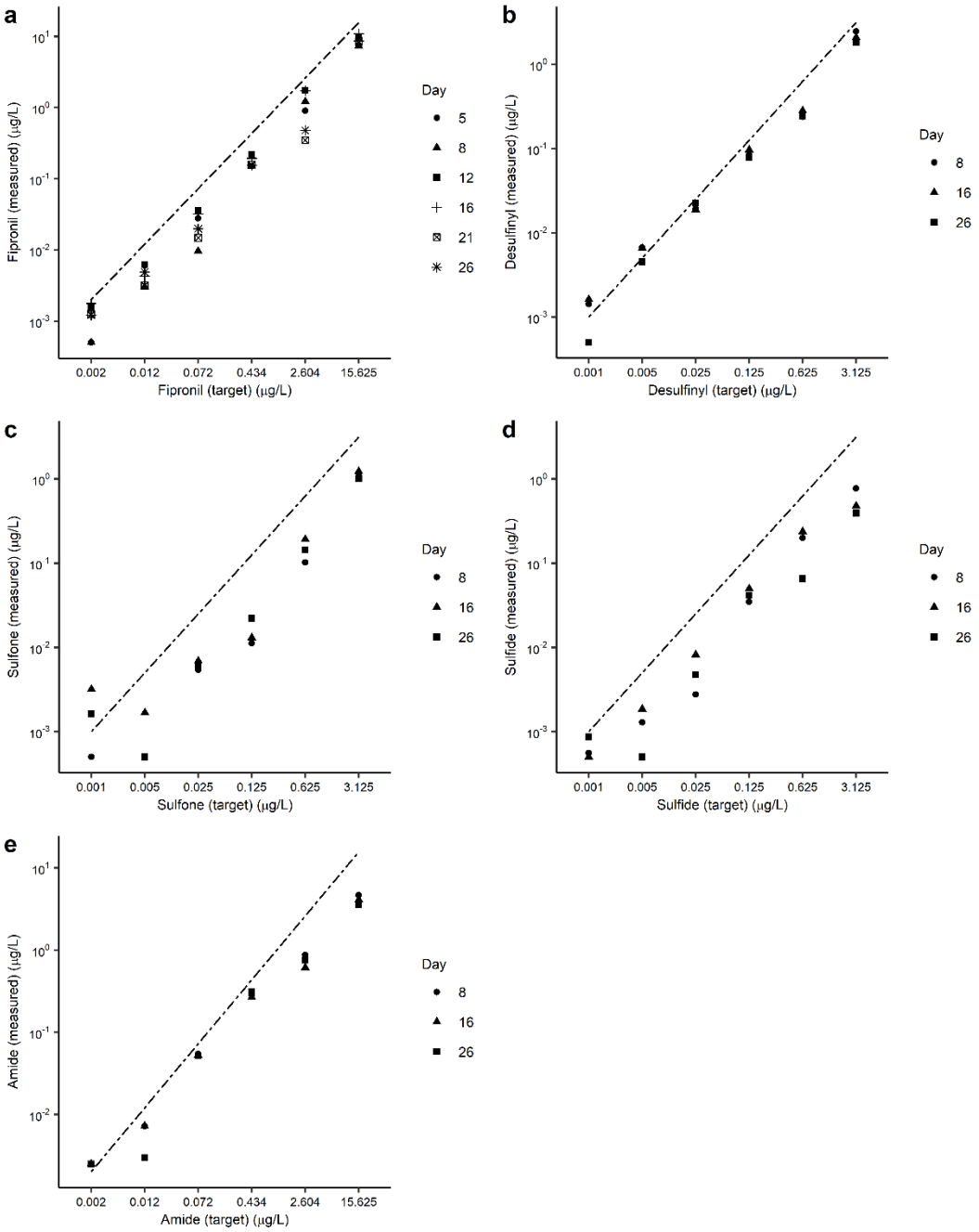

Figure S2. Each measured concentration of (a) fipronil, (b) desulfinyl, (c) sulfone, (d) sulfide, and (e) amide in each treatment plotted by day of experiment. Target concentrations are on the x-axis and measured concentrations on the y-axis (log_{10}). Diagonal is a 1:1 line. By design, concentrations are anticipated to increase over time for the first 4-10-days given daily renewal of a full volume of water in the streams, and all treatments start on day 1.

Table S1. Effect concentrations (EC_{50} and EC_{20}± SE; in µg/L) for benthic macroinvertebrate larvae exposed to fipronil compounds in a 30-day stream mesocosm experiment. Effect concentrations are calculated from a three-parameter logistic model. na- Effects not estimated because of poor fit or lack of a standard error estimate. NSC-Nash-Sutcliff Coefficient. EPT- Ephemeroptera + Plecoptera + Tricoptera.

Taxa order	Taxa	Effect concentration (µg/L)	Fipronil Compound							
			Fipronil ± standard error	NSC	Desulfinyl ± standard error	NSC	Sulfone ± standard error	NSC	Sulfide ± standard error	NSC
Ephemeroptera	**Total mayflies**	**EC_{50}**	0.072 ± 0.088	0.74	0.225 ± 0.106	0.64	0.016 ± 0.007	0.70	0.037 ± 0.017	0.73
		EC_{20}	0.041 ± 0.064		0.187 ± 0.218		0.013 ± 0.011		0.024 ± 0.036	
	Baetis tricaudatus	**EC_{50}**	0.017 ± 0.031	0.49	0.144 ± 0.578	0.32	0.009 ± 0.016	0.53	0.025 ± 0.066	0.49
		EC_{20}	0.014 ± 0.037		0.128 ± 0.423		0.008 ± 0.012		0.019 ± 0.071	
	Diphetor hageni	**EC_{50}**	0.008 ± 0.006	0.71	0.023 ± 0.029	0.60	0.007 ± 0.026	0.58	0.014 ± 0.025	0.51
		EC_{20}	0.005 ± 0.004		0.003 ± 0.006		0.006 ± 0.006		0.007 ± 0.015	
	Drunella grandis	**EC_{50}**	0.016 ± 0.016	0.80	0.237 ± 0.080	0.65	0.015 ± 0.013	0.59	0.029 ± 0.049	0.63
		EC_{20}	0.002 ± 0.003		0.160 ± 0.213		0.007 ± 0.007		0.002 ± 0.008	
	***Ephemerella* sp.**	**EC_{50}**	0.128 ± 0.180	0.54	0.252 ± 0.048	0.41	0.055 ± na	0.52	0.038 ± 0.031	0.56
		EC_{20}	0.098 ± 0.241		0.215 ± 0.231		0.050 ± na		0.028 ± 0.096	
	***Epeorus* sp.**	**EC_{50}**	0.030 ± 0.050	0.66	0.080 ± 2.214	0.45	0.014 ± 0.020	0.54	0.023 ± na	0.37
		EC_{20}	0.022 ± 0.010		0.073 ± 4.359		0.012 ± 0.38		0.021 ± na	
	***Rhithrogena* sp.**	**EC_{50}**	0.013 ± 0.034	0.79	0.011 ± 0.004	0.89	0.002 ± 0.002	0.69	0.004 ± 0.003	0.90
		EC_{20}	0.012 ± 0.030		0.007 ± 0.004		0.002 ± 0.001		0.003 ± 0.007	
	***Paraleptophlebia* sp.**	**EC_{50}**	na		na		0.006 ± 0.002	0.36	0.010 ± 0.022	0.34
		EC_{20}	na		na		0.005 ± 0.009		0.003 ± 0.008	
	Mayfly richness	**EC_{50}**	0.074 ± 0.027	0.96	0.185 ± 0.048	0.88	0.014 ± 0.004	0.96	0.050 ± 0.107	0.90
		EC_{20}	0.009 ± 0.005		0.092 ± 0.042		0.005 ± 0.002		0.037 ± 0.015	
Plecoptera	**Total stoneflies**	**EC_{50}**	0.014 ± 0.092	0.57	0.062 ± 0.065	0.58	0.005 ± 0.008	0.63	0.007 ± 0.021	0.59
		EC_{20}	0.012 ± 0.086		0.049 ± 0.080		0.004 ± 0.010		0.005 ± 0.004	
	Capniidae	**EC_{50}**	0.008 ± 0.010	0.50	0.071 ± 0.117	0.43	0.005 ± 0.004	0.48	0.018 ± 0.962	0.48
		EC_{20}	0.004 ± 0.008		0.059 ± 0.184		0.005 ± 0.007		0.015 ± 0.755	
	***Sweltsa* sp.**	**EC_{50}**	0.005 ± 0.001	0.66	0.002 ± 0.002	0.79	na		0.006 ± 0.005	0.66
		EC_{20}	0.004 ± 0.001		0.0003 ± 0.0006		na		0.004 ± 0.005	
	***Prostoia* sp.**	**EC_{50}**	0.013 ± 0.098	0.55	0.061 ± 0.082	0.57	0.004 ± 0.010	0.62	0.006 ± 0.005	0.58
		EC_{20}	0.012 ± 0.086		0.049 ± 0.099		0.004 ± 0.011		0.004 ± 0.007	
	Pteronarcella badia	**EC_{50}**	0.005 ± 0.003	0.61	0.012 ± 0.020	0.54	0.010 ± na	0.38	na	
		EC_{20}	0.004 ± 0.002		0.002 ± 0.004		0.009 ± na		na	
Trichoptera	***Lepidostoma* sp.**	**EC_{50}**	0.364 ± 4.85	0.56	0.25 ± 0.094	0.51	0.004 ± 0.017	0.47	0.040 ± 0.099	0.36
		EC_{20}	0.312 ± 3.450		0.171 ± 0.36		na		0.001 ± 0.005	
Diptera	***Micropsectra/ Tanytarsus* sp.**	**EC_{50}**	0.248 ± 0.593	0.32	0.36 ± 1.31	0.14	na		na	
		EC_{20}	0.161 ± 0.185		0.27 ± 0.455		na		na	
	***Rheocricotopus* sp.**	**EC_{50}**	0.022 ± 0.011	0.40	0.172 ± 0.370	0.31	0.012± 0.012		0.014 ± 0.272	0.41
		EC_{20}	0.017 ± 0.030		0.148 ± 0.343		0.011 ± 0.015	0.33	0.012 ± 0.202	
	***Tvetenia* sp.**	**EC_{50}**	0.114± 0.444	0.37	na		0.055 ± 0.785	0.37	0.018 ± 0.036	0.45
		EC_{20}	0.093 ± 0.521		na		0.050 ± 0.570		0.009 ± 0.022	
	Diptera richness	**EC_{50}**	0.411 ± 0.282	0.77	1.163 ± 1.088	0.38	0.298 ± na	0.46	0.044 ± 0.028	0.72
		EC_{20}	0.123 ± 0.104		0.450 ± 0.564		0.265 ± na		0.016 ± 0.014	
Other metrics	**Total abundance**	**EC_{50}**	0.025 ± 0.018	0.58	0.173 ± 0.109	0.50	0.013 ± 0.009	0.56	0.031 ± 0.039	0.61
		EC_{20}	0.020 ± 0.031		0.107 ± 0.010		0.006 ± 0.006		0.017 ± 0.055	
	Taxa richness	**EC_{50}**	0.112 ± 0.043	0.94	0.225 ± 0.035	0.90	0.056 ± 0.030	0.88	0.053 ± 0.018	0.92
		EC_{20}	0.02 ± 0.011		0.151 ± 0.073		0.008 ± 0.005		0.014 ± 0.008	
	EPT richness	**EC_{50}**	0.036 ± 0.012	0.96	0.169 ± 0.029	0.94	0.024 ± 0.011	0.92	0.054 ± 0.010	0.95
		EC_{20}	0.007 ± 0.003		0.085 ± 0.029		0.005 ± 0.002		0.028 ± 0.009	

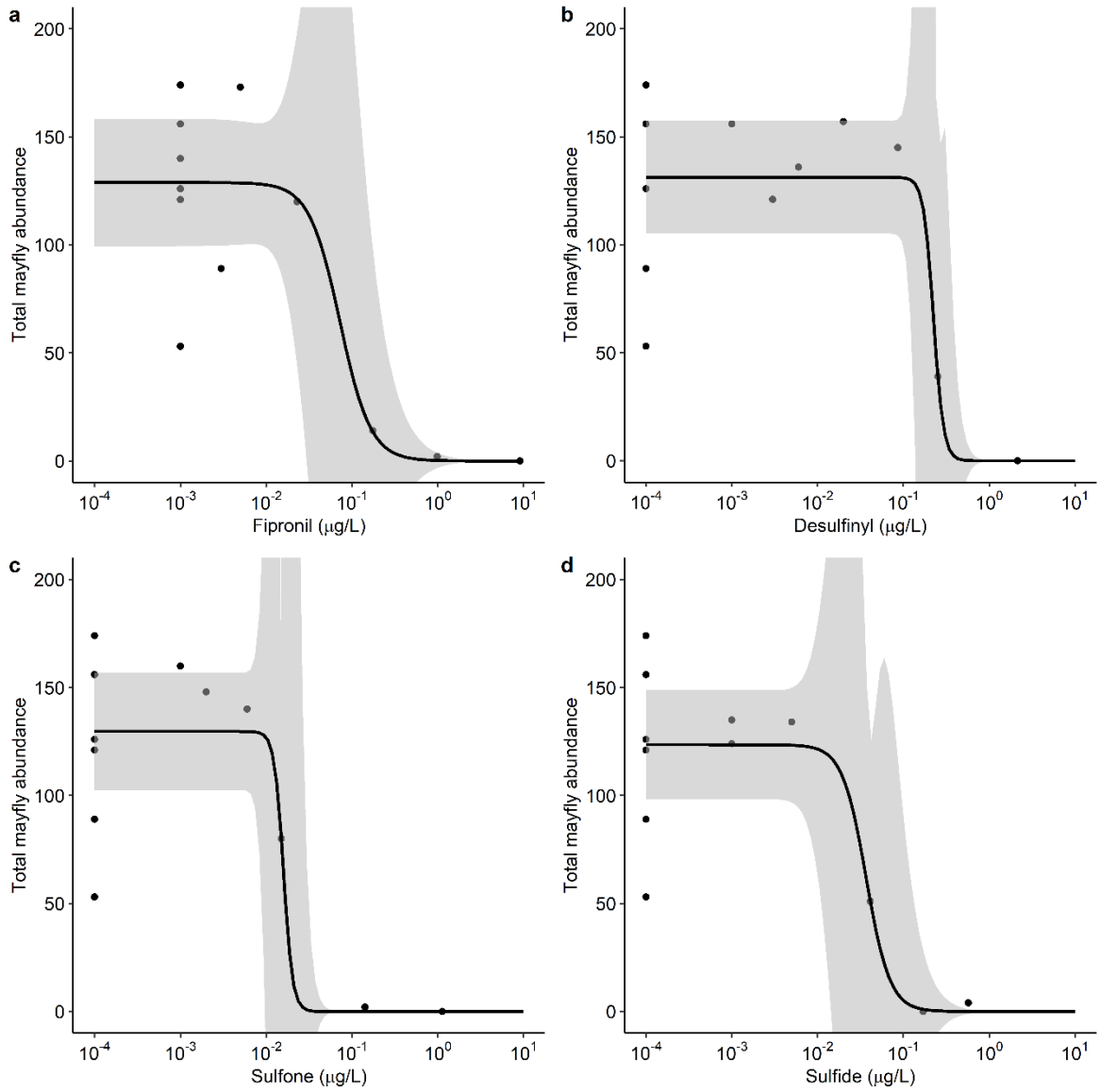

Figure S3. In mesocosm experiments, larval mayfly abundances as a function of (a) fipronil, (b) desulfinyl, (c) sulfone, and (d) sulfide concentration fitted with a three-parameter logistic function. Each data point represents larval abundance from a stream mesocosm at the end of the 30-day experiment. Total mayfly abundance is the sum of mayflies in each stream. Concentration values are time-weighted averages of observed concentrations in each stream mesocosm. Gray ribbon is the 95 percent confidence interval of regression. Note the x-axis is on the log scale.

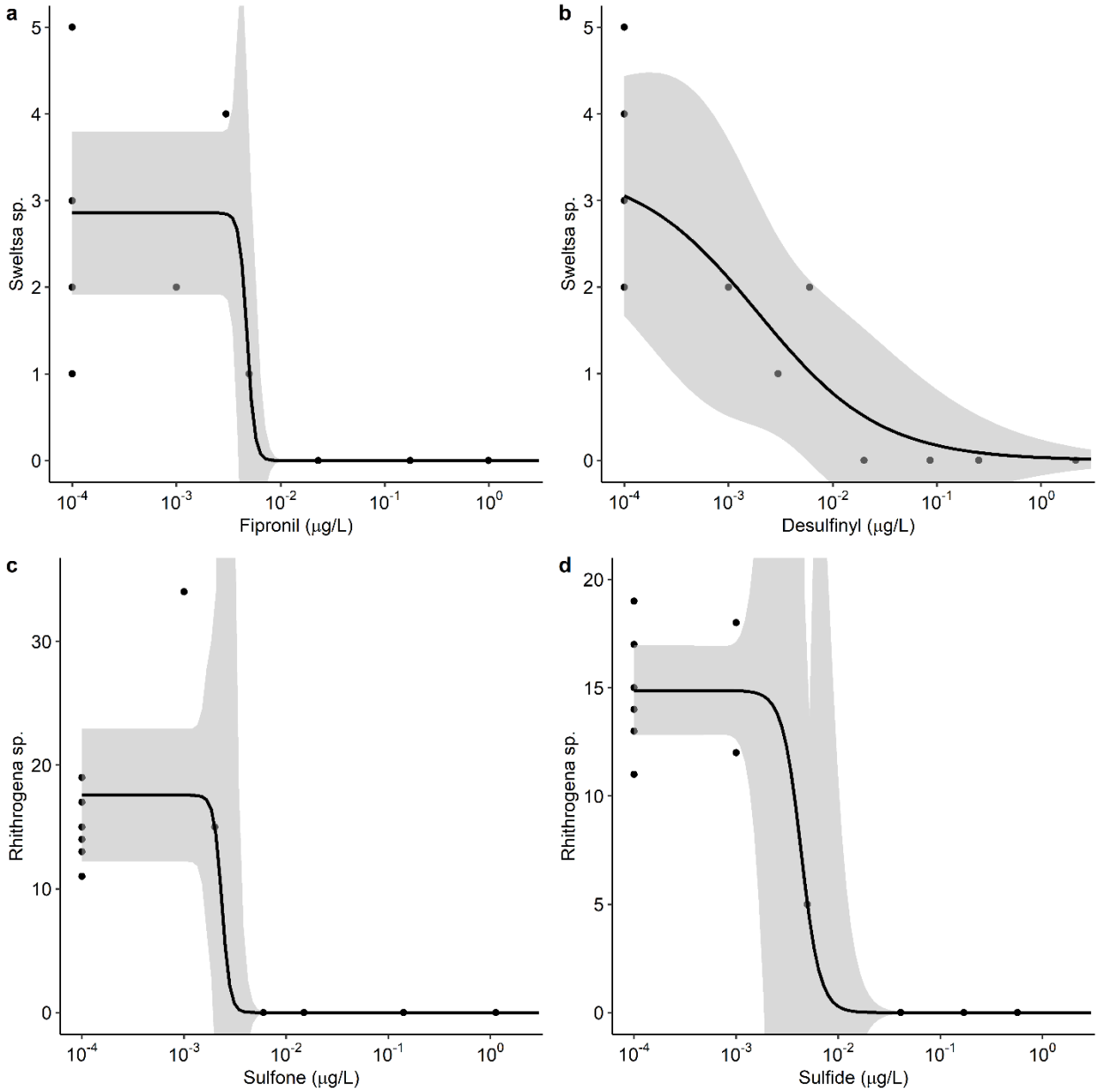

Figure S4. In mesocosm experiments, the most sensitive taxa response to each of the fipronil(s): larval *Sweltsa sp*. abundances versus fipronil (a) and desulfinyl (b), and *Rhithrogena sp*. versus sulfone (c), and sulfide (d). Fitted responses are three-parameter logistic functions. Each data point represents larval abundance from a stream mesocosm at the end of the 30-day experiment. Concentration values are time-weighted averages of observed concentrations in each stream mesocosm. The x-axes are log transformed. Gray ribbon is the 95 percent confidence interval of regression. Note the x-axis is on a log scale.

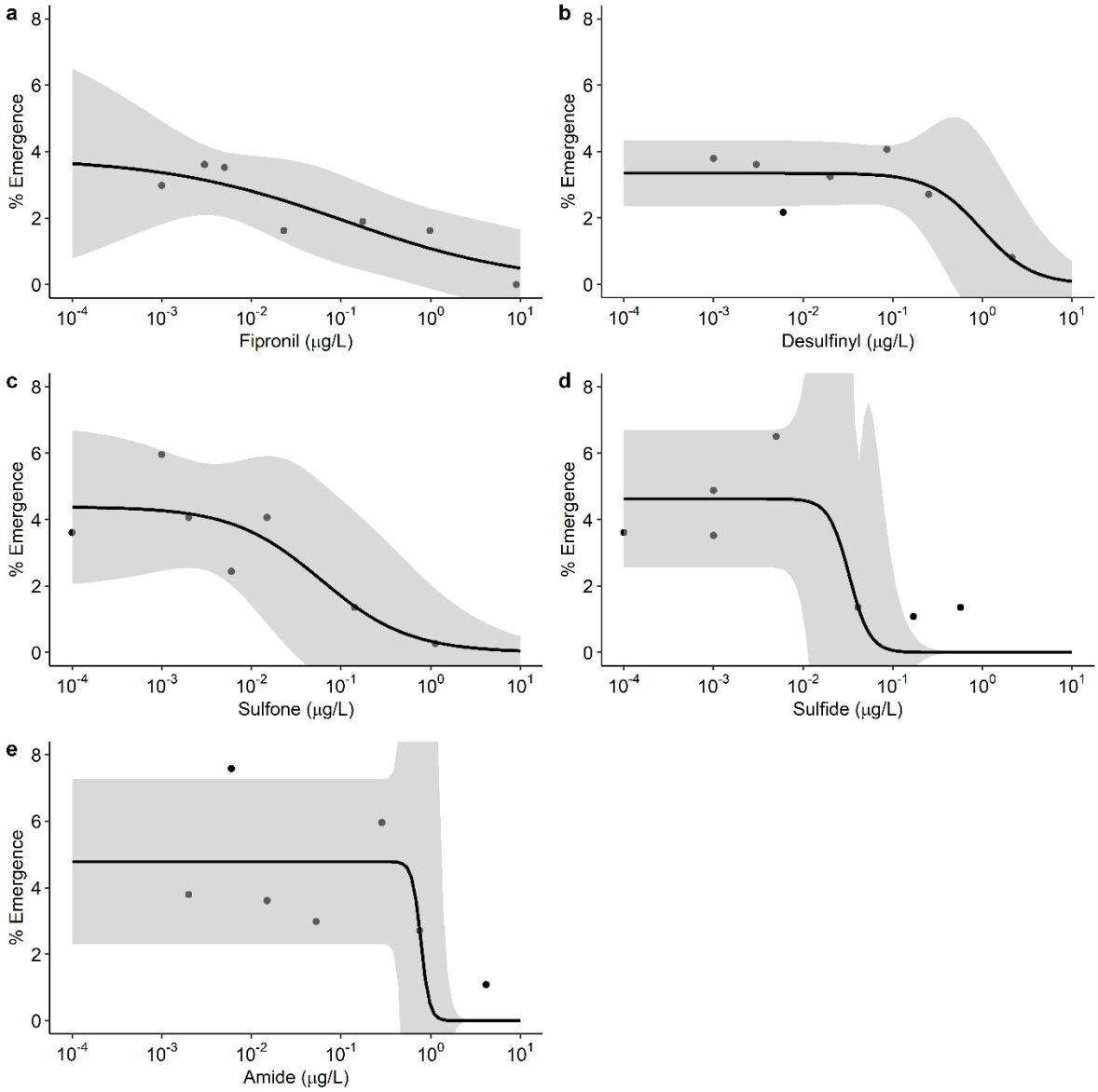

Figure S5. In mesocosm experiments, percent emergence as a function of log-transformed concentration fit with a three-parameter logistic regression for: (a) fipronil, (b) desulfinyl, (c) sulfone, (d) sulfide, and (e) amide. Percent emergence is calculated as the number of emerged adult insects in a stream mesocosm divided by the average number of larvae plus adults in the control stream mesocosms (n=6). Each data point represents an individual stream mesocosm. Concentration values are time-weighted averages of observed concentrations in each stream mesocosm. Gray ribbon is the 95 percent confidence interval for regression. Note the x-axis is on the log scale.

Table S2. Model-fit statistics used for selecting the best-fit data distribution used to estimate Hazard Concentrations at the 5th percentile of each species sensitivity distribution (mesocosm-only data) shown in Figure 4.

Compound	Distribution	Anderson-Darling	Kolmogorov-Smirnov	Cramer-von Mises	Akaike's Information Criterion (AIC)	AIC corrected (AIC_c)	Bayesian Information Criterion	Delta AIC	AIC_c weight
Fipronil	lnorm	0.580	0.189	0.103	-43.2	-42.2	-42.1	1.78	0.262
	lgumbel	0.380	0.167	0.0549	-45.0	-43.8	-43.9	0	0.638
	gamma	0.941	0.268	0.185	-39.4	-38.2	-38.3	5.58	0.039
	weibull	0.760	0.230	0.141	-40.3	-39.1	-39.2	4.67	0.062
Sulfide	lnorm	0.363	0.159	0.0504	-70.6	-69.6	-69.7	0.736	0.239
	lgumbel	0.523	0.183	0.0834	-68.6	-67.7	-67.7	2.65	0.092
	gamma	0.276	0.150	0.0344	-71.2	-70.2	-70.2	0.121	0.325
	weibull	0.258	0.145	0.0308	-71.3	-70.3	-70.3	0	0.334
Desulfinyl	lnorm	0.588	0.202	0.0962	-17.6	-16.3	-16.6	3.16	0.097
	lgumbel	0.764	0.200	0.123	-13.6	-12.3	-12.7	7.11	0.013
	gamma	0.410	0.172	0.0672	-20.8	-19.4	-19.8	0	0.472
	weibull	0.462	0.173	0.0727	-20.5	-19.2	-19.5	0.25	0.417
Sulfone	lnorm	0.377	0.163	0.0460	-73.2	-72.8	-73.2	0.715	0.346
	lgumbel	0.222	0.121	0.0269	-73.9	-73.6	-73.9	0	0.495
	gamma	0.786	0.237	0.123	-70.4	-70.0	-70.4	3.53	0.085
	weibull	0.730	0.231	0.112	-70.1	-69.8	-70.1	3.80	0.074

lnorm-log-normal distribution; lgumbel-log gumbel distribution; gamma-gamma distribution; weibull- weibull distribution

Table S3. Hazard Concentrations for 5% of affected species (HC_5) derived from three datasets: chronic effect concentrations developed in the mesocosm experiment presented here, acute data from the ECOTOX database, and the same data adjusted for exposure duration by dividing by the acute-to-chronic ratio (ACR) of 19.39 (*29*). Numbers in table are mean HC_5 ± standard error and the 5th and 95th percentile confidence intervals.

Dataset	Fipronil	Sulfide	Sulfone	Desulfinyl
Mesocosm-only	4.56 ± 1.99 ng/L (2.59 – 10.2)	3.52 ± 2.02 ng/L (1.36 – 9.20)	2.86 ± 0.86 ng/L (1.93 – 5.29)	3.55 ± 7.79 ng/L (0.35 – 28.4)
Mesocosm and ECOTOX data	5.12 ± 2.02 ng/L (1.97 – 19.0)	4.27 ± 1.28 ng/L (2.39 – 10.10)	2.85 ± 0.87 ng/L (1.46 – 7.14)	4.05 ± 4.14 ng/L (1.49 – 18.9)
Mesocosm and ECOTOX/ACR	4.03 ± 1.93 ng/L (2.11 – 9.61)	3.50 ± 1.02 ng/L (2.32 – 6.29)	2.51 ± 0.68 ng/L (1.69 – 4.31)	4.16 ± 5.79 ng/L (1.05 – 21.7)

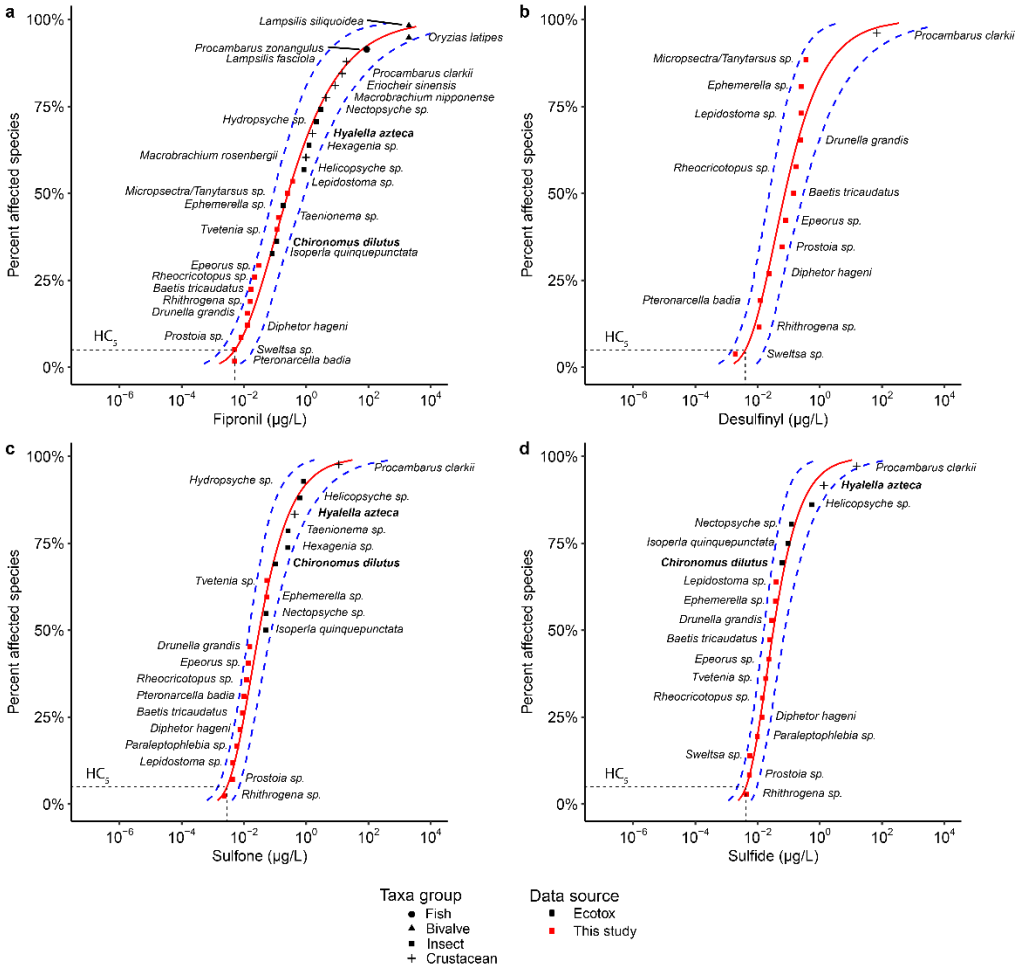

Figure S6. Species sensitivity distributions (SSD) for aquatic invertebrates exposed to (a) fipronil, (b) desulfinyl fipronil, (c) fipronil sulfone, and (d) fipronil sulfide. Data are EC_{50} values derived from the 30-day mesocosm experiment presented here (red symbols) and EC_{50} values (test duration = 4 d, no studies of longer duration were found) from the ECOTOX database (black symbols) (*5, 37-40*). Marker shape indicates taxa group. Blue dashed lines indicate 95% confidence intervals (CI). Horizontal dashed lines indicate the Hazard Concentration for 5% of the species (HC_5). HC_5 values in ng/L derived for each compound were fipronil: 5.12 ng/L (95% CI 1.97–19.0), sulfide: 4.27 ng/L (2.39–10.10), sulfone: 2.85 ng/L (1.46–7.14), and desulfinyl: 4.05 ng/L (1.49–18.9). Bold taxa names indicate taxa discussed in manuscript. Note the x-axis is on the log scale.

Table S4. Model-fit statistics used for selecting the best-fit data distribution used to estimate Hazard Concentrations at the 5th percentile of each species sensitivity distribution (HC_5) observed in Figure S6. Data in the species sensitivity distribution comprised effect concentration (EC_{50}) values from the mesocosm experiment and from the ECOTOX database (*5, 37-40*).

Compound	Distribution	Anderson-Darling	Kolmogorov-Smirnov	Cramer-von Mises	Akaike's Information Criterion (AIC)	AIC corrected (AIC_c)	Bayesian Information Criterion	Delta AIC	AIC_c weight
Fipronil	lnorm	0.543	0.0982	0.00651	116.	116.	118.	4.70	0.087
	lgumbel	0.299	0.100	0.0410	111.	111.	114.	0	0.913
	gamma	3.94	0.290	0.793	142.	142.	145.	30.9	0
	weibull	1.32	0.175	0.188	126.	126.	128.	14.7	0.001
Sulfide	lnorm	0.835	0.196	0.140	-29.9	-29.1	-28.1	6.48	0.038
	lgumbel	0.268	0.115	0.0322	-36.4	-35.6	-34.6	0	0.962
	gamma	2.96	0.357	0.603	-11.3	-10.5	-9.48	-25.1	0
	weibull	1.56	0.225	0.276	-20.9	-20.1	-19.2	15.2	0
Desulfinyl	lnorm	0.618	0.230	0.0924	5.70	6.90	6.83	1.34	0.333
	lgumbel	0.507	0.195	0.0878	4.36	5.56	5.49	0	0.651
	gamma	2.46	0.450	0.523	19.7	20.6	20.8	15.3	0
	weibull	1.25	0.304	0.211	11.7	12.9	12.8	7.35	0.017
Sulfone	lnorm	0.518	0.172	0.0842	-35.0	-34.4	-32.9	3.46	0.15
	lgumbel	0.333	0.133	0.0604	-38.5	-37.8	-36.4	0	0.846
	gamma	2.26	0.264	0.417	-18.7	-18.1	-16.6	19.8	0
	weibull	0.954	0.176	0.143	-27.8	-27.1	-25.7	10.7	0.004

lnorm-log-normal distribution; lgumbel-log gumbel distribution; gamma-gamma distribution; weibull- weibull distribution

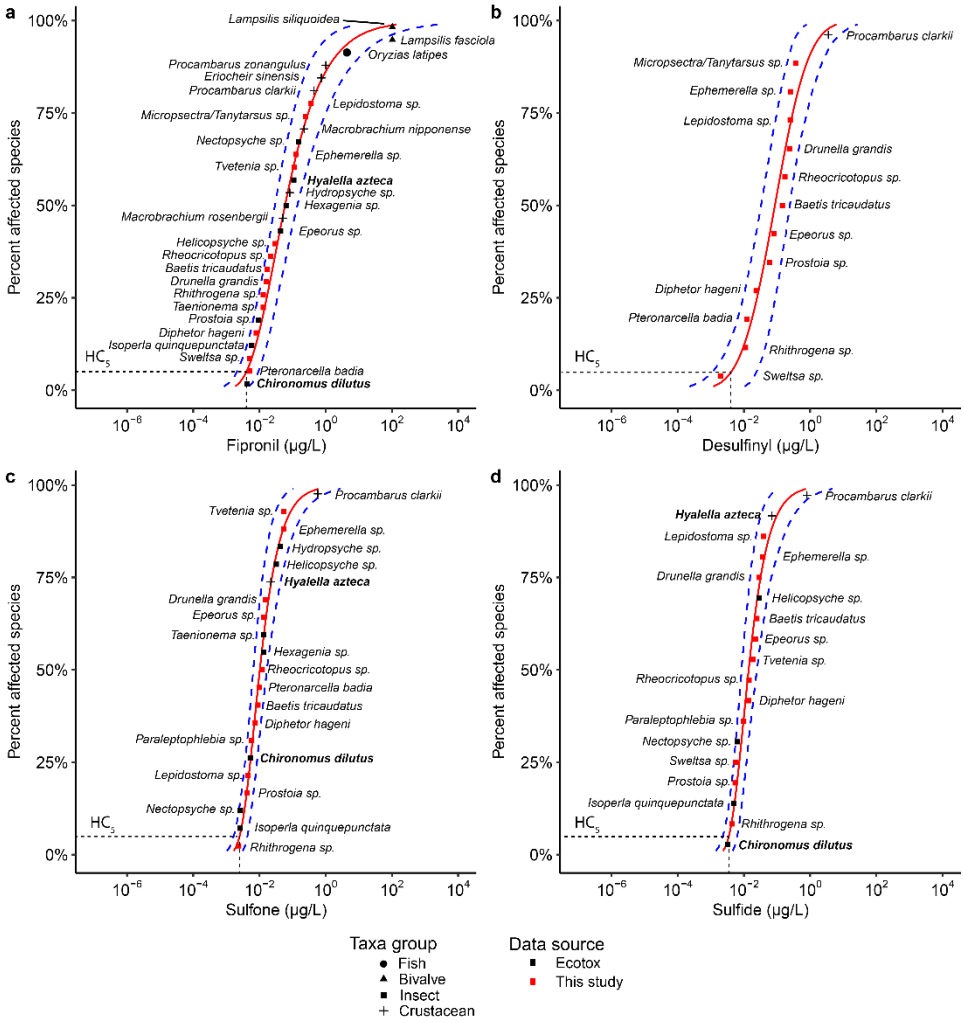

Figure S7. Species sensitivity distributions (SSD) for aquatic invertebrates exposed to (a) fipronil, (b) desulfinyl fipronil, (c) fipronil sulfone, and (d) fipronil sulfide. Data are EC_{50} values derived from the 30-day mesocosm experiment presented here (red symbols) and EC_{50} values (test duration = 4 d, no studies of longer duration were found) from the ECOTOX database (black symbols)(*5, 37-40*) adjusted for exposure duration using the acute to chronic ratio 19.39(*29*). Marker shape indicates taxa group. Blue dashed lines indicate 95% confidence intervals (CI). Horizontal dashed lines indicate the Hazard Concentration for 5% of the species (HC_5). HC_5 values in ng/L derived for each compound were fipronil: 4.03 ng/L (95% CI 2.11–9.61), sulfide: 3.50 ng/L (95% CI 2.32–6.29), sulfone: 2.51 ng/L (95% CI 1.69–4.31), and desulfinyl: 4.16 ng/L (95% CI 1.05–21.7). Note the x-axis is on the log scale. Bold taxa names indicate taxa discussed in manuscript.

Table S5. Model-fit statistics used for selecting the best-fit data distribution used to estimate Hazard Concentrations at the 5th percentile of each Species Sensitivity Distribution (HC_5) observed in Figure S7 (mesocosm data plus data from the ECOTOX database (*5, 37-40*) adjusted for exposure duration using an acute to chronic ratio of 19.39, (*29*)). Data in the Species Sensitivity Distribution included effect concentration (EC_{50}) values from the mesocosm experiment and from the ECOTOX database (*5, 37-40*).

Compound	Distribution	Anderson-Darling	Kolmogorov-Smirnov	Cramer-von Mises	Akaike's Information Criterion (AIC)	AIC corrected (AIC_c)	Bayesian Information Criterion	Delta AIC	AIC_c weight
Fipronil	lnorm	0.908	0.115	0.113	3.84	4.30	6.58	8.55	0.014
	lgumbel	0.288	0.0899	0.0291	-4.71	-4.24	-1.97	0	0.986
	gamma	4.91	0.337	1.01	35.2	35.7	38.0	39.9	0
	weibull	2.15	0.213	0.337	17.5	18.0	20.3	22.2	0
Sulfide	lnorm	0.537	0.144	0.0607	-82.5	-82.5	-80.8	4.46	0.097
	lgumbel	0.342	0.133	0.0544	-87.0	-87.0	-85.2	0	0.902
	gamma	2.27	0.314	0.412	-67.8	-67.8	-66.0	19.3	0
	weibull	1.42	0.227	0.214	-73.2	-73.2	-71.4	13.8	0.001
Desulfinyl	lnorm	0.351	0.149	0.0589	-7.00	-5.80	-5.87	0	0.514
	lgumbel	0.557	0.174	0.0961	-4.50	-3.30	-3.37	2.5	0.147
	gamma	0.782	0.248	0.129	-3.31	-2.11	-2.18	3.69	0.081
	weibull	0.438	0.183	0.0619	-5.63	-4.43	-4.50	1.37	0.258
Sulfone	lnorm	0.460	0.156	0.0606	-110	-109	-108	4.4	0.1
	lgumbel	0.216	0.0836	0.0273	-114	-114	-112	0	0.9
	gamma	2.23	0.273	0.399	-94.2	-93.5	-92.1	20.3	0
	weibull	1.38	0.199	0.209	-99.9	-99.2	-97.8	14.5	0.001

lnorm-log-normal distribution; lgumbel-log gumbel distribution; gamma-gamma distribution; weibull- weibull distribution

Disclaimers: Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

REFERENCES AND NOTES

1. E. S. Bernhardt, E. J. Rosi, M. O. Gessner, Synthetic chemicals as agents of global change. *Front. Ecol. Environ.* **15**, 84–90 (2017).

2. S. Stehle, R. Schulz, Agricultural insecticides threaten surface waters at the global scale. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 5750–5755 (2015).

3. J. Wolfram, S. Stehle, S. Bub, L. L. Petschick, R. Schulz, Meta-analysis of insecticides in United States surface waters: Status and future implications. *Environ. Sci. Technol.* **52**, 14452–14460 (2018).

4. P. C. Van Metre, I. R. Waite, S. Qi, B. Mahler, A. Terando, M. Wieczorek, M. Meador, P. Bradley, C. Journey, T. Schmidt, D. Carlisle, Projected urban growth in the southeastern USA puts small streams at risk. *PLOS ONE* **14**, e0222714 (2019).

5. N. Simon-Delso, V. Amaral-Rogers, L. P. Belzunces, J. M. Bonmatin, M. Chagnon, C. Downs, L. Furlan, D. W. Gibbons, C. Giorio, V. Girolami, D. Goulson, D. P. Kreutzweiser, C. H. Krupke, M. Liess, E. Long, M. McField, P. Mineau, E. A. D. Mitchell, C. A. Morrissey, D. A. Noome, L. Pisa, J. Settele, J. D. Stark, A. Tapparo, H. Van Dyck, J. Van Praagh, J. P. Van der Sluijs, P. R. Whitehorn, M. Wiemers, Systemic insecticides (neonicotinoids and fipronil): Trends, uses, mode of action and metabolites. *Environ. Sci. Pollut. Res.* **22**, 5–34 (2015).

6. P. C. Van Metre, D. A. Alvarez, B. J. Mahler, L. Nowell, M. Sandstrom, P. Moran, Complex mixtures of Pesticides in Midwest U.S. streams indicated by POCIS time-integrating samplers. *Environ. Pollut.* **220**, 431–440 (2017).

7. R. Budd, M. Ensminger, D. Wang, K. S. Goh, Monitoring fipronil and degradates in California surface waters, 2008–2013. *J. Environ. Qual.* **44**, 1233–1240 (2015).

8. U.S. Environmental Protection Agency (U.S. Environmental Protection Agency, 2017); https://epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-and-ecological-risk.

9. C. A. Morrissey, P. Mineau, J. H. Devries, F. Sanchez-Bayo, M. Liess, M. C. Cavallaro, K. Liber, Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review. *Environ. Int.* **74**, 291–303 (2015).

10. D. Gibbons, C. Morrissey, P. Mineau, A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. *Environ. Sci. Pollut. Res.* **22**, 103–118 (2015).

11. U.S. Environmental Protection Agency, *PRD Appendix A: Food/Feed and Non-Food/Non-Feed Uses Considered in Registration Review Work Planning* (U.S. Environmental Protection Agency, 2010).

12. U.S. Environmental Protection Agency, *Problem Formulation for the Environmental Fate and Ecological Risk, Endangered Species and Drinking Water Assessments in Support of the Registration Review of Fipronil* (U.S. Environmental Protection Agency Office of Pesticide Programs, 2011).

13. U.S. Geological Survey, *Estimated Annual Agricultural Pesticide Use*, *Pesticide Use Maps—Fipronil* (U.S. Geological Survey, 2019).

14. S. V. Mize, S. D. Porter, D. K. Demcheck, Influence of fipronil compounds and rice-cultivation land-use intensity on macroinvertebrate communities in streams of southwestern Louisiana, USA. *Environ. Pollut.* **152**, 491–503 (2008).

15. D. P. Weston, M. J. Lydy, Toxicity of the insecticide fipronil and its degradates to benthic macroinvertebrates of urban streams. *Environ. Sci. Technol.* **48**, 1290–1297 (2014).

16. D. P. Weston, D. Chen, M. J. Lydy, Stormwater-related transport of the insecticides bifenthrin, fipronil, imidacloprid, and chlorpyrifos into a tidal wetland, San Francisco Bay, California. *Sci. Total Environ.* **527**, 18–25 (2015).

17. L. H. Nowell, P. W. Moran, T. S. Schmidt, J. E. Norman, N. Nakagaki, M. E. Shoda, B. Mahler, P. C. Van Metre, W. W. Stone, M. W. Sandstrom, M. L. Hladik, Complex mixtures of dissolved pesticides show potential aquatic toxicity in a synoptic study of Midwestern U.S. streams. *Sci. Total Environ.* **613–614**, 1469–1488 (2018).

18. J. Gan, S. Bondarenko, L. Oki, D. Haver, J. X. Li, Occurrence of fipronil and its biologically active derivatives in urban residential runoff. *Environ. Sci. Technol.* **46**, 1489–1495 (2012).

19. W. H. Clements, Small-scale experiments support causal relationships between metal contamination and macroinvertebrate community composition. *Ecol. Appl.* **14**, 954–967 (2004).

20. A. S. Gunasekara, T. Truong, K. S. Goh, F. Spurlock, R. S. Tjeerdema, Environmental fate and toxicology of fipronil. *J. Pestic. Sci.* **32**, 189–199 (2007).

21. J. D. Maul, A. A. Brennan, A. D. Harwood, M. J. Lydy, Effect of sediment-associated pyrethroids, fipronil, and metabolites on *Chironomus tentans* growth rate, body mass, condition index, immobilization, and survival. *Environ. Toxicol. Chem.* **27**, 2582–2590 (2008).

22. K. Lin, D. Haver, L. Oki, J. Gan, Persistence and sorption of fipronil degradates in urban stream sediments. *Environ. Toxicol. Chem.* **28**, 1462–1468 (2009).

23. D. Hainzl, L. M. Cole, J. E. Casida, Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. *Chem. Res. Toxicol.* **11**, 1529–1535 (1998).

24. D. Schlenk, D. B. Huggett, J. Allgood, E. Bennett, J. Rimoldi, A. B. Beeler, D. Block, A. W. Holder, R. Hovinga, P. Bedient, Toxicity of fipronil and its degradation products to *Procambarus* sp.: Field and laboratory studies. *Arch. Environ. Contam. Toxicol.* **41**, 325–332 (2001).

25. D. Hayasaka, T. Korenaga, K. Suzuki, F. Sánchez-Bayo, K. Goka, Differences in susceptibility of five cladoceran species to two systemic insecticides, imidacloprid and fipronil. *Ecotoxicology* **21**, 421–427 (2012).

26. P. M. Chapman, Whole effluent toxicity testing—Usefulness, level of protection, and risk assessment. *Environ. Toxicol. Chem.* **19**, 3–13 (2000).

27. A. U. Conrad, R. J. Fleming, M. Crane, Laboratory and field response of *Chironomus riparius* to a pyrethroid insecticide. *Water Res.* **33**, 1603–1610 (1999).

28. K. D. Kimball, S. A. Levin, Limitations of laboratory bioassays: The need for ecosystem-level testing. *Bioscience* **35**, 165–171 (1985).

29. H. A. Rogers, T. S. Schmidt, B. L. Dabney, M. L. Hladik, B. J. Mahler, P. C. Van Metre, Bifenthrin causes trophic cascade and altered insect emergence in mesocosms: Implications for small streams. *Environ. Sci. Technol.* **50**, 11974–11983 (2016).

30. C. A. Mebane, T. S. Schmidt, J. L. Miller, L. S. Balistrieri, Bioaccumulation and toxicity of cadmium, copper, nickel, and zinc and their mixtures to aquatic insect communities. *Environ. Toxicol. Chem.* **39**, 812–833 (2020).

31. T. S. Schmidt, H. A. Rogers, J. L. Miller, C. A. Mebane, L. S. Balistrieri, Understanding the captivity effect on invertebrate communities transplanted into an experimental stream laboratory. *Environ. Toxicol. Chem.* **37**, 2820–2834 (2018).

32. J. P. Overmyer, B. N. Mason, K. L. Armbrust, Acute toxicity of imidacloprid and fipronil to a nontarget aquatic insect, *Simulium vittatum Zetterstedt* cytospecies IS-7. *Bull. Environ. Contam. Toxicol.* **74**, 872–879 (2005).

33. J. D. Stark, R. I. Vargas, Toxicity and hazard assessment of fipronil to *Daphnia pulex*. *Ecotoxicol. Environ. Saf.* **62**, 11–16 (2005).

34. M. W. Sandstrom, L. K. Kanagy, C. A. Anderson, C. J. Kanagy, Determination of pesticides and pesticide degradates in filtered water by direct aqueous-injection liquid chromatography-tandem mass spectrometry, in *National Field Manual for the Collection of Water-Quality Data: U.S. Geological Survey Techniques of Water-Resources Investigations No. Chapter B11* (U.S. Geological Survey, 2015).

35. J. L. Miller, T. S. Schmidt, M. W. Sandstrom, P. C. Van Metre, B. J. Mahler, L. H. Nowell, D. M. Carlisle, P. W. Moran, “Dataset for an ecological risk assessment of Fipronil compounds in U.S. streams” (Data release, U.S. Geological Survey, 2020).

36. J. D. Garrett, J. W. Frey, P. C. Van Metre, C. A. Journey, N. Nakagaki, D. T. Button, L. H. Nowell, “Design and methods of the Midwest stream quality assessment (MSQA), 2013” (No. Open-File Report 2017–1073, U.S. Geological Survey, 2017).

37. C. A. Journey, P. C. Van Metre, A. H. Bell, J. D. Garrett, D. T. Button, N. Nakagaki, S. L. Qi, P. M. Bradley, “Design and methods of the southeast stream quality assessment (SESQA), 2014” (No. Open-File Report 2015–1095, U.S. Geological Survey, 2015).

38. R. W. Sheibley, J. L. Morace, C. A. Journey, P. C. Van Metre, A. H. Bell, N. Nakagaki, D. T. Button, S. L. Qi, “Design and methods of the Pacific Northwest Stream Quality Assessment (PNSQA), 2015” (No. Open-File Report 2017–1103, U.S. Geological Survey, 2017).

39. J. F. Coles, K. Riva-Murray, P. C. Van Metre, D. T. Button, A. H. Bell, S. L. Qi, C. A. Journey, R. W. Sheibley, “Design and methods of the US Geological Survey Northeast Stream Quality Assessment (NESQA), 2016” (No. Open-File Report 2018–1183, U.S. Geological Survey, 2019).

40. J. T. May, L. H. Nowell, J. F. Coles, D. T. Button, A. H. Bell, S. L. Qi, P. C. Van Metre, “Design and methods of the California Stream Quality Assessment (CSQA), 2017” (No. Open-File Report 2020–1023, U.S. Geological Survey, 2020).

41. U.S. Geological Survey, “Chapter A4. Collection of water samples” (Techniques of Water-Resources Investigations 09-A4, U.S. Geological Survey, 2006).

42. M. W. Sandstrom, F. D. Wilde, “Syringe-filter procedure for processing samples for analysis of organic compounds by DAI LC-MS/MS” (National Field Manual for the Collection of Water-Quality Data: U.S. Geological Survey Techniques of Water-Resources Investigations No. Chapter 5.2.2.B, U.S. Geological Survey, 2014).

43. R Development Core Team (R Foundation for Statistical Computing, 2015).

44. C. Ritz, F. Baty, J. C. Streibig, D. Gerhard, Dose-response analysis using R. *PLOS ONE* **10**, e0146021 (2015).

45. J. Nash, J. V. Sutcliffe, River flow forecasting through conceptual models, I: A discussion of principles. *J. Hydrol.* **10**, 398–409 (1970).

46. J. S. Lefcheck, piecewiseSEM: Piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods Ecol. Evol.* **7**, 573–579 (2016).

47. J. B. Grace, *Structural Equation Modeling and Natural Systems* (Cambridge Univ. Press, 2006).

48. J. Thorley, C. Schwarz, ssdtools: An R package to fit species sensitivity distributions. *J. Open Source Softw.* **3**, 1082 (2018).

49. M. A. Beketov, K. Foit, R. B. Schäfer, C. A. Schriever, A. Sacchi, E. Capri, J. Biggs, C. Wells, M. Liess, SPEAR indicates pesticide effects in streams—Comparative use of species- and family-level biomonitoring data. *Environ. Pollut.* **157**, 1841–1848 (2009).

50. M. Liess, R. B. Schäfer, C. A. Schriever, The footprint of pesticide stress in communities–species traits reveal community effects of toxicants. *Sci. Total Environ.* **406**, 484–490 (2008).

51. J. J. Rasmussen, A. Baattrup-Pedersen, S. E. Larsen, B. Kronvang, Local physical habitat quality cloud the effect of predicted pesticide runoff from agricultural land in Danish streams. *J. Environ. Monit.* **13**, 943–950 (2011).

52. S. N. Wood, Generalized additive models: An introduction with R (2nd edition). *J. Stat. Softw.* **86** (2018).

53. M. D. Poteat, D. B. Buchwalter, Four reasons why traditional metal toxicity testing with aquatic insects is irrelevant. *Environ. Sci. Technol.* **48**, 887–888 (2014).

54. N. Eisenhauer, A. Bonn, C. A. Guerra, Recognizing the quiet extinction of invertebrates. *Nat. Commun.* **10**, 50 (2019).

55. J. M. Kraus, T. S. Schmidt, D. M. Walters, R. B. Wanty, R. E. Zuellig, R. E. Wolf, Cross-ecosystem impacts of stream pollution reduce resource and contaminant flux to riparian food webs. *Ecol. Appl.* **24**, 235–243 (2014).

56. T. S. Schmidt, J. M. Kraus, D. M. Walters, R. B. Wanty, Emergence flux declines disproportionately to larval density along a stream metals gradient. *Environ. Sci. Technol.* **47**, 8784–8792 (2013).

57. I. R. Waite, M. D. Munn, P. W. Moran, C. P. Konrad, L. H. Nowell, M. R. Meador, P. C. Van Metre, D. M. Carlisle, Effects of urban multi-stressors on three stream biotic assemblages. *Sci. Total Environ.* **660**, 1472–1485 (2019).

58. C. J. Walsh, A. H. Roy, J. W. Feminella, P. D. Cottingham, P. M. Groffman, R. P. Morgan II, The urban stream syndrome: Current knowledge and the search for a cure. *J. North Am. Benthol. Soc.* **24**, 706–723 (2005).

59. J. L. Meyer, M. J. Paul, W. K. Taulbee, Stream ecosystem function in urbanizing landscapes. *J. North Am. Benthol. Soc.* **24**, 602–612 (2005).

60. M. A. Beketov, B. J. Kefford, R. B. Schäfer, M. Liess, Pesticides reduce regional biodiversity of stream invertebrates. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 11039–11043 (2013).

61. T. S. Schmidt, P. C. Van Metre, D. M. Carlisle, Linking the agricultural landscape of the Midwest to stream health with structural equation modeling. *Environ. Sci. Technol.* **53**, 452–462 (2019).

62. I. R. Waite, P. C. Van Metre, Multistressor predictive models of invertebrate condition in the Corn Belt, USA. *Freshw. Sci.* **36**, 901–914 (2017).

63. C. Moschet, I. Wittmer, J. Simovic, M. Junghans, A. Piazzoli, H. Singer, C. Stamm, C. Leu, J. Hollender, How a complete pesticide screening changes the assessment of surface water quality. *Environ. Sci. Technol.* **48**, 5423–5432 (2014).

64. bbe-Moldaenke, *BenthoTorch User Manual* (bbe-Moldaenke, 2013).

65. U.S. Environmental Protection Agency, “National rivers and streams assessment 2008–2009” (Technical Report EPA/841/R-16/008, 2016).

66. W. J. Gerth, A. T. Herlihy, Effect of sampling different habitat types in regional macroinvertebrate bioassessment surveys. *J. North Am. Benthol. Soc.* **25**, 501–512 (2006).

67. O. Mohseni, H. G. Stefan, T. R. Erickson, A nonlinear regression model for weekly stream temperatures. *Water Resour. Res.* **34**, 2685–2692 (1998).

68. A. C. Benke, A. D. Huryn, L. A. Smock, J. B. Wallace, Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *J. North Am. Benthol. Soc.* **18**, 308–343 (1999).

69. K. P. Burnham, D. R. Anderson, *Model Selection and Multimodal Inference: A Practical Information-Theoretic Approach* (Springer-Verlag, ed. 2, 2002).

70. S. Knillmann, P. Orlinskiy, O. Kaske, K. Foit, M. Liess, Indication of pesticide effects and recolonization in streams. *Sci. Total Environ.* **630**, 1619–1627 (2018).

71. Agilent Technologies, *Agilent MassHunter Workstation Software. Quantitative Analysis Familarization Guide* (Agilent Technologies Inc., 2010).