Environmental Science
Water Research & Technology

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Journal:	*Environmental Science: Water Research & Technology*
Manuscript ID	EW-ART-03-2022-000157
Article Type:	Paper

Modeling Risk Dynamics of Contaminants of Emerging Concern in a Temperate-region Wastewater Effluent-dominated Stream

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Abstract

Wastewater effluent-dominated streams are becoming increasingly common worldwide, including in temperate regions, with potential impacts on ecological systems and drinking water sources. We recently quantified the occurrence/ spatiotemporal dynamics of pharmaceutical mixtures in a representative temperate-region wastewater effluent-dominated stream (Muddy Creek, Iowa) under baseflow conditions and characterized relevant fate processes. Herein, we quantified the ecological risk quotients (RQs) of 19 effluent-derived contaminants of emerging concern (CECs; including: 14 pharmaceuticals, 2 industrial chemicals, and 3 neonicotinoid insecticides) and 1 run-off-derived compound (atrazine) in the stream under baseflow conditions, and estimated the probabilistic risks of effluent-derived CECs under all-flow conditions (i.e., including runoff events) using stochastic risk modeling. We determined that 11 out of 20 CECs pose medium-to-high risks to local ecological systems (i.e., algae, invertebrates, fish) based on literature-derived acute effects under measured baseflow conditions. Stochastic risk modeling indicated decreased, but still problematic, risk of effluent-derived CECs (i.e., RQ≥0.1) under all-flow conditions when runoff events were included. Dilution of effluent-derived chemicals from storm flows thus only minimally decreased risk to aquatic biota in the effluent-dominated stream. We also modeled in-stream transport. Thirteen out of 14 pharmaceuticals persisted along the stream reach (median attenuation rate constant $k<0.1\ h^{-1}$) and entered the Iowa River at elevated concentrations. Predicted and measured concentrations in the drinking water treatment plant were below the human health benchmarks. This study demonstrates the application of probabilistic risk assessments for effluent-derived CECs in a representative effluent-dominated stream under variable flow conditions (when measurements are less practical) and provides an enhanced prediction tool transferable to other effluent-dominated systems.

Water Impact Statement

We used chemical and continuous flow data for stochastic risk modeling to demonstrate that risks to aquatic biota from effluent-derived chemicals decrease only minimally when diluted with storm flows. Stochastic risk modeling is useful for assessments when chemical data are limited but flow data are available. This work is generalizable to effluent-dominated systems critical for *defacto* water reuse management decisions.

1. Introduction

Climate change and urbanization are increasing the influence of wastewater effluent on receiving waters, leading to an increase in contaminants of emerging concern (CECs) loadings including pharmaceuticals, personal care products, pesticides, and industrial chemicals from wastewater to drinking water supplies (i.e., de facto reuse).[1–6] Because some CECs such as pharmaceuticals and pesticides are biologically active at low concentrations by design and have potential to accumulate in aquatic and terrestrial species,[7–9] occurrence of these contaminants in drinking water, irrigation water, and food webs may pose risks/concerns to wildlife and human health.[10–14] For example, neonicotinoid insecticides (i.e., clothianidin, imidacloprid, thiamethoxam) and more human-toxic metabolites have been found in finished drinking water (i.e., tap water);[10,15,16] potential concerns include inflammation of the liver and central nervous system due to chronic exposure.[17] Pharmaceuticals such as metformin (antidiabetic) and venlafaxine (antidepressant) can cause behavior changes,[18] potential endocrine disruption effects,[19,20] and reduced size and fecundity in fish.[21] Despite several studies that suggest negligible adverse effects of different CECs to humans,[22,23] knowledge is limited for chronic effects via long-term exposure or exposure to complex contaminant mixtures.[24] Effluent-dominated streams, where treated wastewater represents the majority of flow, can represent a 'worst-case scenario' for risk assessment of different CEC mixtures under baseflow conditions, but characterizing the potential risks to biota under elevated flow conditions (i.e., including events with surface runoff that dilute wastewater influence) is important to reflect real-world conditions.

For a robust assessment of the potential risks of CECs in effluent-dominated systems, high spatiotemporal-resolution sampling synergized with comprehensive analytical analysis and

the application of appropriate simulation models are imperative. The risk quotient (RQ), expressed as the ratio of the measured environmental concentration (MEC) to the predicted no effect concentration (PNEC), is often used for risk characterization of ecological systems.[25] Numerous studies have quantified the occurrence and distribution of different CECs and associated RQs in the aquatic environment;[5,26–28] however, environmental variability makes assessing risk dynamics of CECs under variable conditions challenging (i.e., logistically difficult, expensive). Stochastic risk modeling has been used for assessing risk at contaminated sites under various input sources and hydrologic conditions.[29,30] Stochastic approaches apply probability distributions to describe random variability in input parameters; these distributions are then propagated to the output variables through mathematical models using statistical sampling algorithms.[29] For example, a Monte Carlo simulation is an effective approach for characterizing risks and uncertainty where a considerable amount of data describing the system dynamics is available.[30] Although such simulations do not account for possible interactive effects to organisms (e.g., antagonistic, synergistic interactions) from chemical mixtures, stochastic risk modeling can serve as an important tool for probabilistically assessing the contaminant risk and identifying dominant risk drivers.[31]

The spatial and temporal heterogeneity of environmental variables controlling attenuation processes makes investigation of CECs in-stream transport challenging. Thus, a simulation approach can help integrate varied environmental conditions (e.g., hydrologic conditions, microbial activity, etc.) and quantitative information to predict the transport of CECs under various flow conditions that generate chronic exposure to aquatic biota with changing spatial and temporal dynamics.[32] QUAL2K is a one-dimensional stream water quality model intended to

represent a well-mixed channel[33] that does not require extensive data inputs beyond basic first-order kinetic rates,[34–36] and is commonly applied.[34,37]

Our prior work demonstrated that Muddy Creek (Coralville, IA) is a representative effluent-dominated stream in temperate region, and it is also an ideal study site where we conducted long-term monitoring of chemical and hydrologic data.[38–42] In the previous work, we quantified the occurrence/ spatiotemporal dynamics and fate mechanisms of pharmaceutical mixtures[38,43] and neonicotinoid insecticides[39] in an effluent-dominated stream under baseflow conditions. Nevertheless, the potential risks to the local ecological system under all-flow conditions and the drinking water source have not yet been evaluated. One may assume dilution of WWTP effluent-derived CECs from run-off events would substantially decrease overall risks to biota, but comprehensively evaluating exposure can be difficult because capturing samples under variable flow conditions is inherently more logistically onerous than measuring at baseflow. Therefore, appropriate simulation approaches can fill this knowledge gap and help develop a comprehensive risk assessment for pharmaceuticals and other CECs in this representative effluent-dominated stream to provide an enhanced prediction tool transferable to other effluent-dominated systems.

The present study objectives were to: (1) quantify the ecological exposure risks of pharmaceuticals and other CECs in an effluent-dominated stream and assess changes in risk exposure for effluent-derived chemicals simulated under variable all-flow conditions due to dilution with storm flows; and (2) estimate the in-stream transport and input of pharmaceuticals and other CECs from the effluent-dominated stream to a drinking water source and their potential exposure risks to human health. We hypothesized that: (1) the in-stream ecological risk of effluent-derived CECs was lower under non-baseflow conditions, but CECs can still pose

risks to aquatic biota; and (2) CECs from the effluent-dominated stream posed minimal risks to drinking water intakes following substantial dilution after entering the larger waterbody. Herein, we demonstrate a novel framework to characterize exposure risks of aquatic biota from effluent-derived chemicals in an effluent-dominated stream under variable flow conditions. We use collected chemical data and real-time flows data for stochastic risk modeling to demonstrate that risks to aquatic biota from effluent-derived chemicals decrease only minimally when diluted with storm flows. Stochastic risk modeling helps inform temporal risk dynamics and transport modeling informs spatial attenuation dynamics. The present study integrated our previously released chemical data[38,39,42] for simulation and risk assessment and collected new chemical data to quantify intra-day variability of CECs in the stream and potential impacts on the drinking water intakes.

2. Materials and Methods

2.1 Study site

Muddy Creek, Iowa, USA (Latitude 41°42'00", Longitude 91°33'46") has a drainage area of 22.5 km^2 composed of both agricultural (17.45%–20.72%) and urban (60%–72.5%) land use (details in Table S.1), and discharges into the Iowa River (Figure 1). The long-term median flow during two years (September 2017 to August 2019) of Muddy Creek (station ID 05454090)[41] and Iowa River at Iowa City (station ID 05454500)[44] was 0.18±1.14 m^3/s (median±standard deviation) and 74±103 m^3/s, respectively. The mixing ratio of Muddy Creek stream flow to Iowa River flow was roughly 1:411 based on the long-term median flow discharge. Muddy Creek is a wastewater effluent-dominated stream, with effluent discharged from the North Liberty Wastewater Treatment Plant (WWTP). North Liberty, Iowa, has an estimated population[45] of 19,240 and is the second-fastest growing city in Iowa. The WWTP has a modern membrane

bioreactor facility built in 2008, which removes particles >0.02 μm and thus no further disinfection is used. This facility also implements biological nitrogen and phosphorus removal. The current wastewater discharge averages approximately 5,300 m^3/day (0.061 m^3/s).[46] The Muddy Creek streamflow varied from 0.03 m^3/s to 0.30 m^3/s (median 0.12 m^3/s) at the sampling time points during 2 years of baseflow sample collection (at U.S. Geological Survey [USGS] gaging station 05454090; DS2).[38,41] Muddy Creek has a generally sandy streambed and heavy tree canopy riparian zone. Four USGS sampling sites were established for this study: (1) 0.1 km upstream from WWTP outfall (US1; station ID 05454050); (2) wastewater effluent outfall (Effluent; station ID 05454051); (3) 0.1 km downstream from WWTP outfall (DS1; station ID 05454052); and (4) 5.1 km downstream from WWTP outfall (DS2, USGS gaging station; station ID 05454090) (Figure 1a). The estimated distance from DS2 to the Iowa River is roughly 2 km.

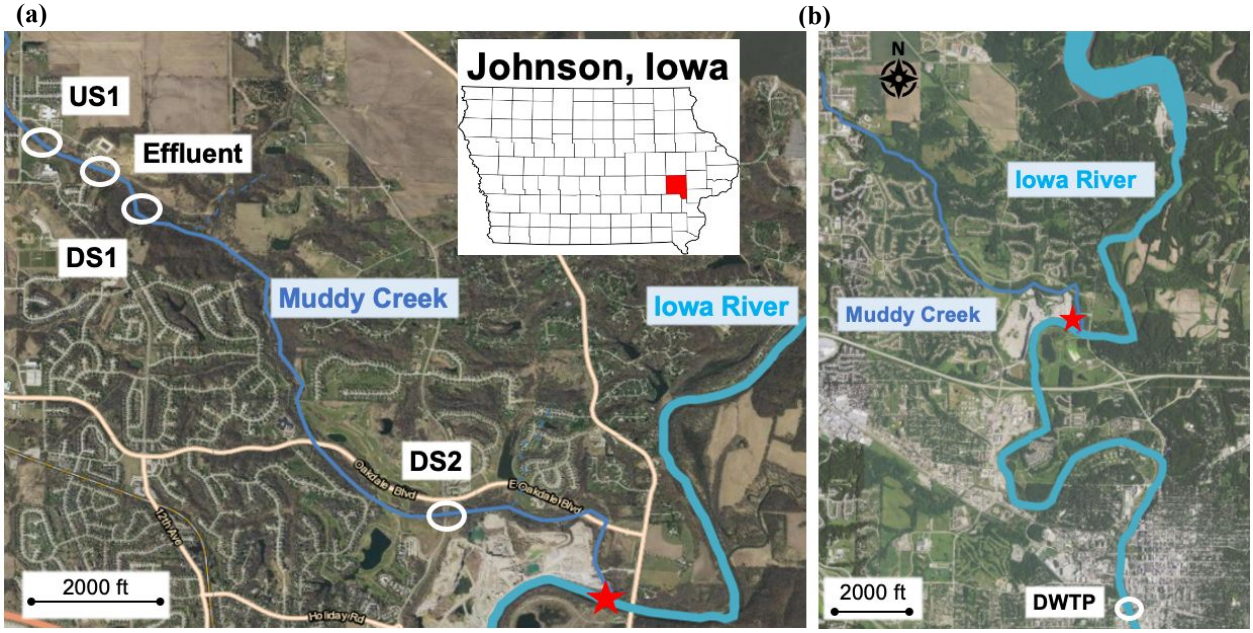

Figure 1: (a) Sampling map of Muddy Creek, Coralville, Johnson County, Iowa, USA. The sampling location values include: US1 (station ID 05454050, 0.1 km upstream from the wastewater treatment plant (WWTP) outfall), Effluent (station ID 0545405, wastewater effluent outfall), DS1 (station ID 05454052, 0.1 km downstream from WWTP outfall) and DS2 (USGS gaging station, station ID 05454090, Latitude 41°42'00", Longitude 91°33'46", 5.1 km downstream from WWTP outfall). (b) Sampling map of drinking water treatment plant, roughly 8km downstream of where Muddy Creek enters the Iowa River. The red star represents where Muddy Creek joins the Iowa River. Base map is from Iowa Geographic Map Server.[47]

2.2 Data Sources

Hydrologic data. During monthly sampling events (September 2017 to August 2018), streamflow at US1 and DS1 was measured via a flow tracker using established USGS methods.[48] Effluent discharge at the specific time of sampling was determined indirectly by subtracting the streamflow measured above from that measured below the WWTP effluent. Stream stage at DS2, located 5.1 km downstream from the effluent outfall, was continuously monitored by the USGS gaging station (station ID 05454090) to calculate flow based on a stage/discharge rating curve developed for this specific site (Figure S.3).[41] Baseflow discharge and all-flow discharge at DS2 during 2-year period were 0.137±0.067 m^3/s (mean±standard deviation) and 0.372±1.142 m^3/s, respectively.[41] Bulk water quality parameters including dissolved oxygen, pH, water temperature, and conductivity were also monitored by USGS for the monthly sampling time points during Year 1.[42]

Chemical data. Chemical data sources consisted of previously released data and additional newly collected data. Previously reported data of 20 CECs included: 1) Monthly pharmaceutical data (n=14 compounds; 12 data sets in total; September 2017-August 2018; "Year 1" of the study; Table S.2) collected and analyzed by USGS,[42] used to simulate the first-order attenuation; and monthly pharmaceutical data (n=14 compounds; 12 data sets in total; September 2017-August 2018; "Year 1"; Table S.2) collected and analyzed by University of Iowa (UIowa), used to validate the attenuation simulation;[38] 2) Neonicotinoid insecticide data (n=3 compounds; 17 data sets in total; collected approximately twice-monthly during weather-dependent baseflow conditions; September 2018-August 2019; "Year 2"; Table S.2) were collected and analyzed by UIowa;[39] and 3) Other chemicals (n=3) including atrazine and benzotriazole and 5-methyl-benzotriazole (Table S.2).[38,42] All chemical data from Year 1 that are described above were used

for risk assessment (but only effluent derived chemical were used for stochastic risk modeling; see below). Each data set included four samples from each of our four established sampling sites. All the sampling procedures, sample pretreatments and analytical methods were fully described in our prior publications.[38,39] Although the occurrence of 20 CECs from Muddy Creek was released previously,[38,39] no prior ecological and human risk assessments have been conducted.

New to this study, additional water samples were collected over a more-intensive four-day period in the wastewater effluent and along the stream reach to capture a higher resolution of daily variation of CECs (i.e., 14 pharmaceuticals and 2 industrial chemicals). During July 14-18, 2019 (a total of 96 h), sampling occurred three time daily (8am, 12pm, and 7pm) at four sampling sites [US1, Effluent, DS1, and DS2] to capture the intra- and inter-daily variation of CECs in the effluent and along the stream reach. A one-time water sampling event also occurred at the UIowa drinking water treatment plant (DWTP; roughly 8km distance downstream from where Muddy Creek enters the Iowa River; Figure 1b) where raw and finished drinking water was sampled to evaluate potential impacts of Muddy Creek on a local drinking water source. Detailed analytical method and quality assurance/quality control (QA/QC) were previously published.[38,39] Chemicals are fully described in Table S.3.

2.3 Risk assessment

The potential risks of CECs in the effluent dominated stream to aquatic organisms were assessed using a risk quotient (RQ), calculated as the ratio of the measured environmental concentration (MEC) to the predicted no effect concentration (PNEC; based on cited literature values) of a target compound for 3 different aquatic organism types: algae, invertebrates, and fish (Eqn. 1; Table S.4-S.7).[25] The measured concentration along the stream reach during the 2-year

period (including all the monitored pharmaceuticals, pesticides, and industrial chemicals from three in-stream sites: US1, DS1, and DS2) was used to generate the MEC value. For the ecological risk assessment, the measured chemical data from the different stream sites were pooled due to the relatively short stream reach (~5.2km, where some aquatic species such as fish living in the stream could swim freely throughout the reach) and thus the study reach was effectively treated as a single 'site' for the purposes of risk analysis. We recognize that this spatial simplification has limitations, particularly for less mobile aquatic organisms that do not move throughout the reach. The Effluent site was not considered an "in-stream site" because this was an outfall pipe above the stream where effluent is discharged into the stream and thus no biota directly inhabits this site. For acute toxicity, the lowest values of half-maximal effective concentration (EC50) or half-lethal concentration (LC50) divided by an assessment factor (AF) of 1,000 corresponds to the PNEC acute value (Eqn. 2; Table S.4). For chronic toxicity, no observed effect concentrations (NOEC) or lowest observed effect concentrations (LOEC) were used and the applied AF value was 10.[49] If different toxicity data were available for the same species from the database, the lowest value was chosen to provide a conservative assessment (Table S.5, S.7).

$$\mathrm{RQ} = \frac{MEC}{PNEC} \qquad \text{Equation (1)}$$

$$\mathrm{PNEC} = \frac{\mathrm{EC50\ or\ LC50}}{AF} \text{ for acute toxicity} \qquad \text{Equation (2)}$$

$$\mathrm{PNEC} = \frac{\mathrm{NOEC\ or\ LOEC}}{AF} \text{ for chronic toxicity} \qquad \text{Equation (3)}$$

Commonly-used ranking criteria[32,50] were adopted in this work: RQ≥1, high ecotoxicological risk; 0.1≤RQ<1, medium ecotoxicological risk; RQ<0.1, low ecotoxicological risk.

2.4 Stochastic risk modeling

Risk Quotients (RQs) were calculated based on measured chemical data and flow data under baseflow conditions; however, uncertainties and variabilities exist when considering all-flow conditions for risk characterization in the deterministic method. Thus, a stochastic risk approach can be useful, whereby risk output is a probability distribution. Risk uncertainty was considered by conducting Monte Carlo simulations using Minitab (version 19). Individual compounds were selected for stochastic risk analysis when the 75th percentile of the total measured RQs under baseflow conditions exceeded the lowest problematic risk level (i.e., RQ=0.1) for at least one of the three different aquatic biota types. For pharmaceuticals, RQs generated from acute toxicity data were used for risk modeling purpose, whereas for industrial chemicals and pesticides, both RQs generated from acute toxicity and chronic toxicity were applied and discussed in the present study; decisions were based on acute/chronic data availability.

In Monte Carlo simulations, each random variable is defined by a probability distribution with a corresponding mean and a standard deviation. First we selected compounds based on when the 75^{th} percentile RQ exceeded the lowest problematic risk level (i.e., RQ=0.1) for at least one of the three different aquatic species types (Figure 3, S.6) under baseflow conditions. For acute effects 10 compounds (bupropion, citalopram, tramadol, sulfamethoxazole, desvenlafaxine, lidocaine, methocarbamol, imidicloprid, clothianidin and thiamethoxam) were selected and for chronic effects, 3 compounds (imidacloprid, clothianidin and thiamethoxam) were selected. We then examined the log-normality distribution of the RQs calculated from all our monitoring chemical data (from the in-stream sites US1, DS1 and DS2 during 2-year period) via Kolmogorov-Smirnov test. All compounds except lidocaine, citalopram, and thiamethoxam selected for the risk analysis passed the log normality test (i.e., were not significantly different

206 from a log-normal distribution, α=0.05); thus, we considered this distribution model a valid
207 approximation. Flow discharge at DS2 at the time of sampling events were used to characterize
208 long-term baseflow conditions.[41] Continuous flow discharge (every 15 min) at DS2 during the 2-
209 year period (over which the chemical samples were taken) was used to characterize the "all-flow"
210 conditions.[41] The flow data under baseflow conditions and all-flow conditions at DS2 also passed
211 the log normality test. Although we only had one site (DS2) with long-term continuously
212 monitored flow data from the USGS gaging station, the flow variation at DS2 is representative of
213 the hydrologic dilution factor conditions for the stream reach. This assumption is reasonable for
214 contaminants primarily originating from the point-source WWTP (i.e., effluent-derived) because
215 overland flow contributions of pharmaceuticals are expected to be minimal; we recognize that
216 this assumption has limits for substances contributed by nonpoint sources such as many
217 pesticides (previously, however, we demonstrated that neonicotinoid insecticides in the stream
218 are driven by contributions of wastewater).[39]

219 For stochastic risk modeling, we treated the entire stream reach as a single segment; thus,
220 we included RQ data from all three in-stream sites (US1, DS1 and DS2) under baseflow
221 conditions to generate the probabilistic distribution. Although this can neglect spatial differences
222 within the segment, based on the relatively short distance (~5.2km) that permits aquatic species
223 such as fish to readily move freely within the stream, this approach appeared reasonable to
224 generate an average risk distribution in the stream reach (we recognize that limited-mobility
225 organisms likely move less within the segment). This assumption is also reasonable for
226 evaluating changes in risk from effluent-derived chemicals under different storm flow conditions
227 in an effluent-dominated stream, but may not be applicable under all scenarios. Based on our
228 recent work probing attenuation mechanisms in the stream,[43] the single-segment assumption is

likely reasonable because most compounds persist except for citalopram, which rapidly sorbs to bed sediments and concentrations change greatly along the stream reach even over short distances. The corresponding mean and standard deviation of RQs under baseflow conditions and flow discharge from baseflow and all-flow conditions, respectively, were used to generate probabilistic distributions for each input variable (i.e., baseflow RQ, baseflow discharge, and all-flow discharge). The output was a probabilistic distribution of RQs under all-flow conditions. The Monte Carlo simulation performed 1,000 iterations for each variable considered to ensure numerical stability. The Monte Carlo simulation workflow (using pharmaceutical fexofenadine as an example) is shown in Figure S.4. Detailed input values for Monte Carlo simulations are shown in Tables S.9, S.10, and S.13.

2.5 Attenuation modeling in the stream

Based on our prior investigation demonstrating that Muddy Creek is well-mixed laterally and vertically,[38] the QUAL2K model is appropriate to simulate the attenuation dynamics of CECs in the effluent-dominated stream used as a study reach. Transport modeling of effluent-derived CECs (i.e., 14 pharmaceuticals) was conducted using the QUAL2K software (version 2.07) to simulate multiple CECs along the stream reach that were mainly discharged from the wastewater effluent. The QUAL2K software was developed by the United States Environment Protection Agency (USEPA). It consists of an Excel workbook (QUAL2K.xls) that provides the user interface to the model and a Fortran executable (Q2KFortran2_04.exe) that runs the calculations. Full details of QUAL2K and its use are described in the QUAL2K User Guide.[33] The model allows users to segment the stream into several reaches and further divide each reach into a series of equally spaced elements, which are fundamental computational units of the model.

Muddy Creek is a relatively small stream during baseflow conditions (0.137±0.067 m^3/s; mean±standard deviation) and does not contain substantial contributing branches, thus a mainstem with four segments was used for simulation (Figure S.5) A steady-state flow balance is implemented for each model element. The QUAL2K model allows specification of the many kinetic parameters on a reach-specific basis, such as a chemical attenuation rate based on the first-order kinetics, which makes it suitable for CEC simulations in the effluent-dominated stream.[34,51,52]

The input hydraulic data and chemical data for this study were based on field measurements (see SI; Table S.15, Figure S.9). First-order kinetics (Eqn. 4) were used for target CECs based on the QUAL2K model.

$$\frac{C}{C0} = e^{-kt} \qquad \text{Equation (4)}$$

Rate constant (k) and half-life ($t_{1/2}$) values for individual compounds were calculated based on the USGS monthly data (chemical and discharge) in the effluent and at both downstream sites (DS1 and DS2) during Year 1 (Table S.15, S.16). The monthly chemical data measured by UIowa at the corresponding sites were used for model validation. Upstream site US1 was excluded because the primary source of pharmaceuticals[38] (and most neonicotinoids[39]) to this system was almost completely derived from the wastewater effluent. The initial concentration in the effluent was multiplied by an immediate dilution factor (Eqn. 4) due to the dilution by upstream flow.

$$\text{Immediate dilution factor} = \frac{\text{Effluent flow rate}}{\text{Effluent flow rate} + \text{Upstream flow rate}} \qquad \text{Equation (5)}$$

Risk assessment was conducted by comparing the predicted CEC concentrations to human health benchmark values at the point where Muddy Creek enters the Iowa River.

3. Results and Discussion

3.1 Ecological risks quantification and stochastic modeling

We demonstrated that the suite of CECs measured were consistently released to the receiving water from the WWTP, indicating our long-term monitoring data source was representative for risk assessments under baseflow conditions. During a higher-resolution short-period monitoring (4 day; three times per day) of the pharmaceuticals and industrial chemicals in the effluent and along the stream reach, we observed reasonably consistent intra-day concentrations (Effluent: within 86–124% variability; DS1: 46–119%; DS2: 73–117%) and compositions of CECs (Figure 2), indicating that a single sample within a day was representative of daily loadings under baseflow conditions. We did observe inter-day concentration variability between weekday and weekend samples (Figure 2), demonstrating the value of long-term field monitoring to capture temporal variation under baseflow conditions. For example, the total pharmaceutical concentrations on Sunday were roughly 50% lower than those on weekdays. During this time, the daily flow from the WWTP was stable and consistent (between 0.08-0.09 m^3/s; Figure S.2). This was only one short-period sampling campaign during a single season (summer); however, similar weekday/weekend pharmaceutical variations have been reported previously elsewhere.[53] Although pharmaceuticals constituted the highest concentrations and were regularly released from the WWTPs into Muddy Creek, our present and prior studies demonstrated unknown upstream sources combined with wastewater effluent contributed pesticides and industrial chemicals to the stream,[38,39,42] creating more-complex evolving CEC mixtures along the stream with potential implications to aquatic biota. Despite the continuously high inputs of pharmaceuticals and industrial chemicals, research on chronic and acute effects

299 are limited compared to pesticides.[54–58] For example, previous research indicated that the acute
300 toxic pressure was mainly driven by pesticides including clothianidin in a wastewater-impacted
301 stream, while the total concentration sums downstream were clearly dominated by
302 pharmaceuticals or other household chemicals.[59] Nevertheless, long-term chronic effects from
303 high levels of pharmaceuticals and other household chemicals are still poorly understood.[59] Thus,
304 more long-term baseflow exposure and associated toxicity data for pharmaceutical mixtures is
305 warranted for a more comprehensive risk assessment in effluent-dominated streams.

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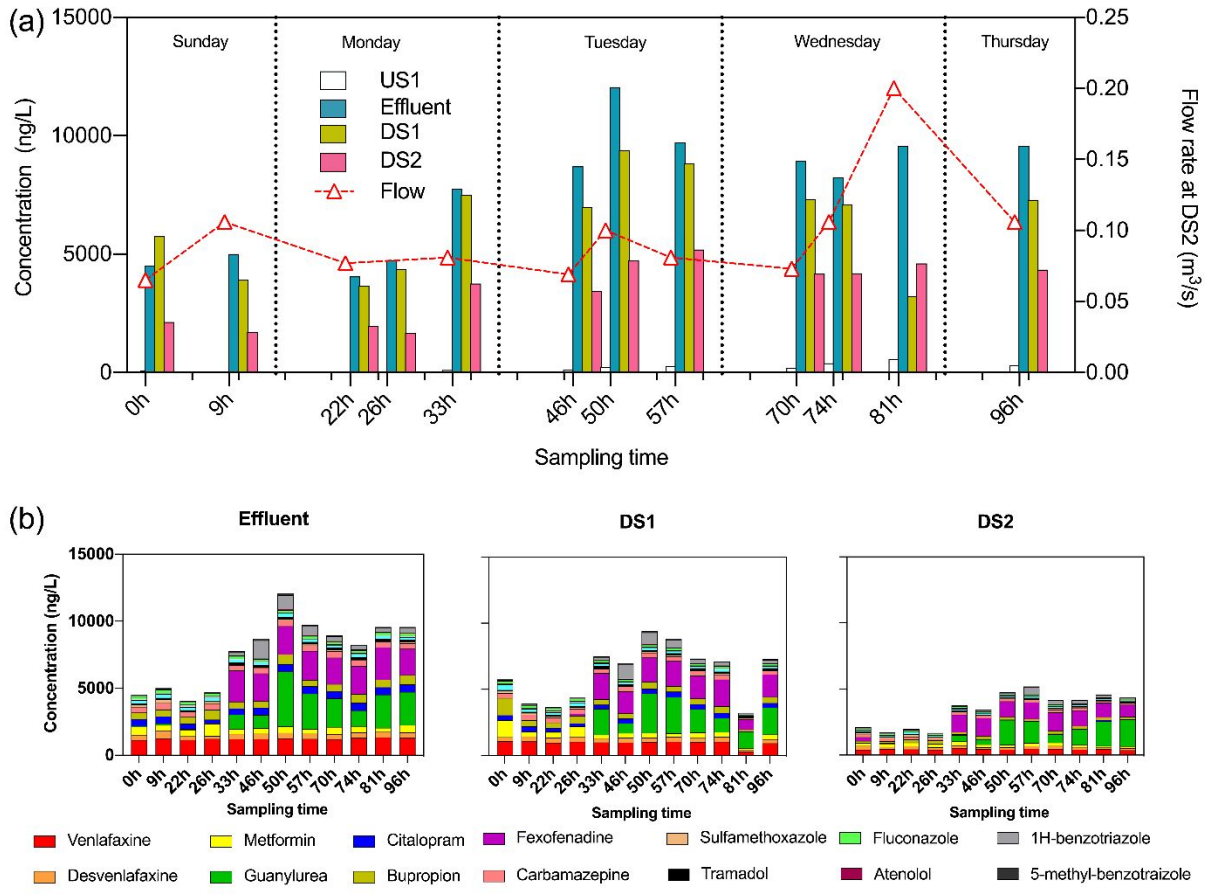
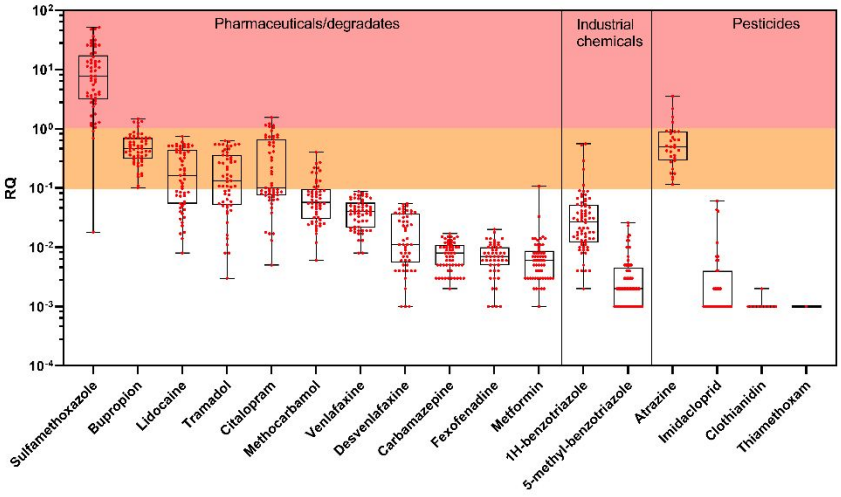


308
309 **Figure 2:** Daily total (a) and individual (b) concentration variations of pharmaceuticals and industrial chemicals
310 during 4-day period (total=96h, sampling events n=12) at three sampling sites [Effluent, DS1, DS2] in Muddy Creek
311 (Coralville, Iowa). Sampling occurred between 10am July 14, 2019 and 10am July 18, 2019.
312

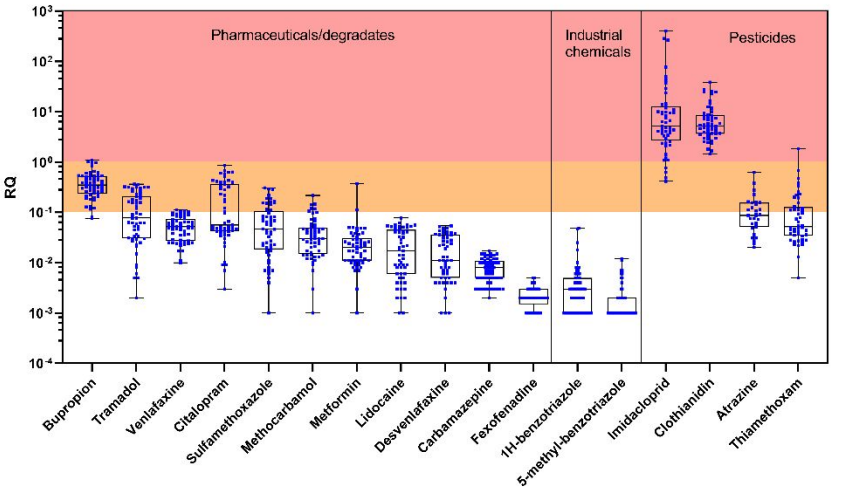
We quantified the RQs (based on acute effects) for different CECs in the stream under baseflow conditions, and demonstrated that 11 out of 18 CECs (two CECs do not have toxicity data available) can pose medium to high risks to local ecological systems (i.e., within the stream). For algae, sulfamethoxazole can pose high risks (RQ≥1) under baseflow conditions, and five CECs including 4 pharmaceuticals (bupropion, lidocaine, tramadol, and citalopram) and 1 pesticide (atrazine) can pose medium risks (0.1≤RQ<1) under baseflow conditions (Figure 3). For invertebrates, 2 pesticides (clothianidin and imidacloprid) can pose high risks under baseflow conditions and 5 CECs can pose medium risks, whereas the other CECs pose minimal risks under baseflow conditions. Although imidacloprid mainly originated from the WWTP effluent,[39] atrazine was mainly present in the upstream runoff and not present in the effluent;[38] thus, effluent effectively diluted atrazine under baseflow conditions and decreased the risks posed by atrazine. Moreover, only 2 CECs (methocarbamol and desvenlafaxine) exhibited medium risks to fish, whereas all other CECs exhibited minimal risks. Despite the common occurrence and/or high concentrations of pharmaceutical transformation product (i.e., guanylurea), their toxicity data are not available and consequently RQs could not be determined; transformation products may pose additional presently unquantified risks. We also quantified the RQs of chronic effects for industrial chemicals and pesticides including neonicotinoids and atrazine, and the results demonstrated that imidacloprid and clothianidin can pose medium to high risks to algae and invertebrate, respectively, whereas other chemicals exhibited minimum risks (Figure S.6). Risk assessment for both acute and chronic effects are critical to comprehensively evaluate the ecological risks of CECs in Muddy Creek, however, chronic toxicity data for pharmaceuticals are generally lacking due to regulatory requirements for pharmaceuticals worldwide and limited data access.

When assessing the environmental risk of mixtures, substantial knowledge gaps exist on the mechanisms and drug-drug interactions of pharmaceuticals and their metabolites in non-target organisms. Although single-compound risk assessments are crucial to identifying key risk drivers, aquatic biota globally are exposed to CEC mixtures that may affect each taxonomic group differently. Thus, risk assessments to specific taxonomic groups, such as fish, crustaceans, and algae using the concentration addition model have been developed and reported in the literature to estimate the cumulative risks.[32] Nevertheless, the simultaneous presence of different CECs can result in not only additive effects, but also synergistic and antagonistic toxic effects at concentrations lower than the PNEC for each individual compound;[60] thus, considering the interactive effects of CEC mixtures makes risk assessment inherently tenuous. For example, toxicity tests exposing aquatic organisms to combinations of various pharmaceuticals including carbamazepine, diclofenac, and ibuprofen revealed stronger effects than what would be expected singly.[61,62] Therefore, summing up individual RQs may be an overly simplified approach to estimate the risk of a mixture and indeed may underestimate the synergistic or antagonistic effects from CEC mixtures.[63,64]

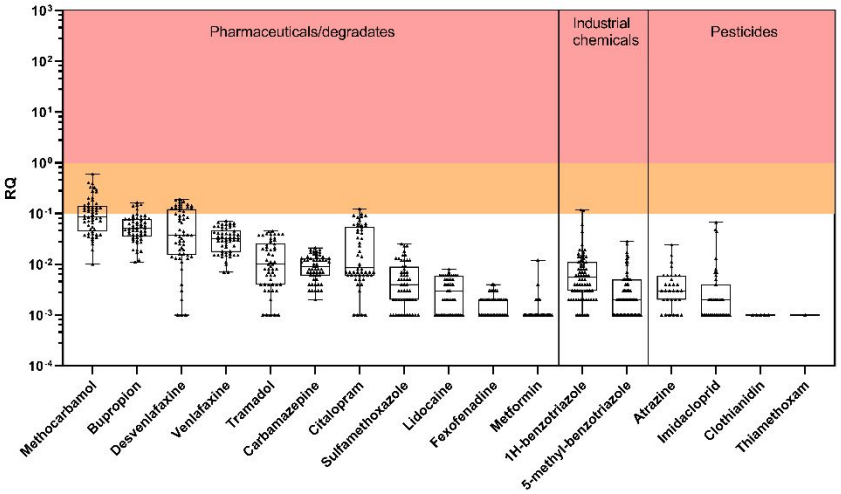
(a) Algae



(b) Invertebrate



(c) Fish



351

Figure 3: Measured risk quotients (RQs) for algae (a), invertebrates (b) and fish (c) of contaminants of emerging concern (CECs) aggregated from all three in-stream sites (US1, DS1, DS2) based on acute toxicity data in Muddy Creek. RQs $<10^{-4}$ were considered negligible risks and were not included in the figure. Atenolol was excluded in (a-c) due to all RQs were $<10^{-4}$. No risk assessment data are available for fluconazole and guanylurea due to a lack of literature on toxicity. Red shade indicates high risk (RQ≥1), orange shade indicates medium risk (0.1≤RQ<1), no shade indicates low risks. The box and whiskers from bottom to top represent minimum value, 25th percentile, median value, 75th percentile and maximum value.

The presence of CECs under both baseflow conditions and elevated flow conditions (i.e., runoff events) generated chronic exposure to aquatic species with changing dynamics; thus, it is critical to assess risk comprehensively under all-flow conditions. We demonstrated decreasing risk of effluent-derived CECs (14 pharmaceuticals, 2 industrial chemicals and 3 neonicotinoids) under all-flow conditions via stochastic risk modeling based on acute and chronic toxicity data, which covers a broader range of conditions than baseflow alone and can help us better understand the dynamics of effluent-dominated streams integrated with environmental uncertainties. Compounds were selected for stochastic risk simulation when at the 75th percentile of the total measured RQs under baseflow conditions exceeded the lowest problematic risk level (i.e., RQ=0.1) for at least one of the three different aquatic species types (Figure 3, S.6).

Our results of the all-flows simulation demonstrate that compounds that posed medium-to-high risks under baseflow conditions were still problematic (i.e., RQ≥0.1) when runoff events were included (Figure 4, S.7; Table S.11, S.14). For acute effects, 9 out of 11 CECs still pose medium to high risks to at least one of the three different aquatic species (Figure 4; Table S.11), whereas 2 out of 3 neonicotinoids can pose medium to high risks for chronic effects (Figure S.7; Table S.14). Although the 'worst-case' risk exposure conditions can be conservatively characterized under baseflow conditions, our stochastic simulation results indicate that lower frequency runoff events do not substantially decrease the potential risks of the effluent-derived CECs we measured (0.21-0.59 fold-change for acute RQ; 0.17-0.95 fold-change for chronic RQ).

In other words, dilution due to storm flows does not meaningfully decrease risk to aquatic biota from effluent-derived chemicals in the effluent-dominated stream. Our stochastic risk assessment was predicated on the assumption that during elevated flow conditions, RQs would decrease mainly due to dilution; this is very reasonable for a point-source of contaminants that are less frequently present in overland flow. This assumption, however, may underestimate non-point source pesticides and other CECs that are transported by overland flow (e.g., atrazine mainly from the upstream sources,[38] PAHs from stormwater runoff,[65] etc.).

Indeed, our approach is limited to risks associated from effluent-derived chemicals. Nevertheless, because Muddy Creek is a relatively small watershed with mixed agricultural (17.45%) and urban (60%) land use (Table S.1), we expect more contaminants to enter Muddy Creek from urban non-point sources (e.g., heavy metals, urban-use pesticides) rather than from agricultural non-point sources (e.g., atrazine, clothianidin) during runoff events. Additionally, some agricultural pesticides such as clothianidin (solubility in water: 0.327 g/L) and atrazine (0.0347 g/L) can leach into agricultural drainage tiles, particularly post-application, and enter streams under baseflow conditions.[66–68] In prior work, we demonstrated that the WWTP is a significant, year-round point-source of imidacloprid but that imidacloprid also has some upstream origins;[39] thus, this pesticide could be present from both point and nonpoint sources in the watershed. The composition of pharmaceutical mixtures can also reportedly be affected by flow conditions (e.g., carbamazepine dominated under baseflow conditions and caffeine dominated in flood events).[32] Therefore, we recognize the limits to the stochastic risk model and the primary utility in estimating changing risk dynamics for the effluent-derived chemicals (e.g., pharmaceuticals) in this study reach. Nevertheless, the mixed-use watershed across an agricultural to urban gradient and the variety of potential non-point source contributions of

chemicals means we also cannot necessarily assume simple changes in risk dynamics under changing flows (e.g., that risk of agricultural pesticides automatically increases under elevated flow conditions). The described modeling approach is very useful for evaluating changes in exposure risk associated with effluent-derived chemicals under variable flow conditions, including dilution of effluent by storm flows in an effluent-dominated stream. This approach is ideal when there may be limitations to the quantity of chemical data, but there is sufficient flow characterization (as is common in continuously gaged streams). Stochastic risk modeling can provide an important basis for changing risk conditions especially in effluent-dominated streams, even if there are limits to the scope of application.

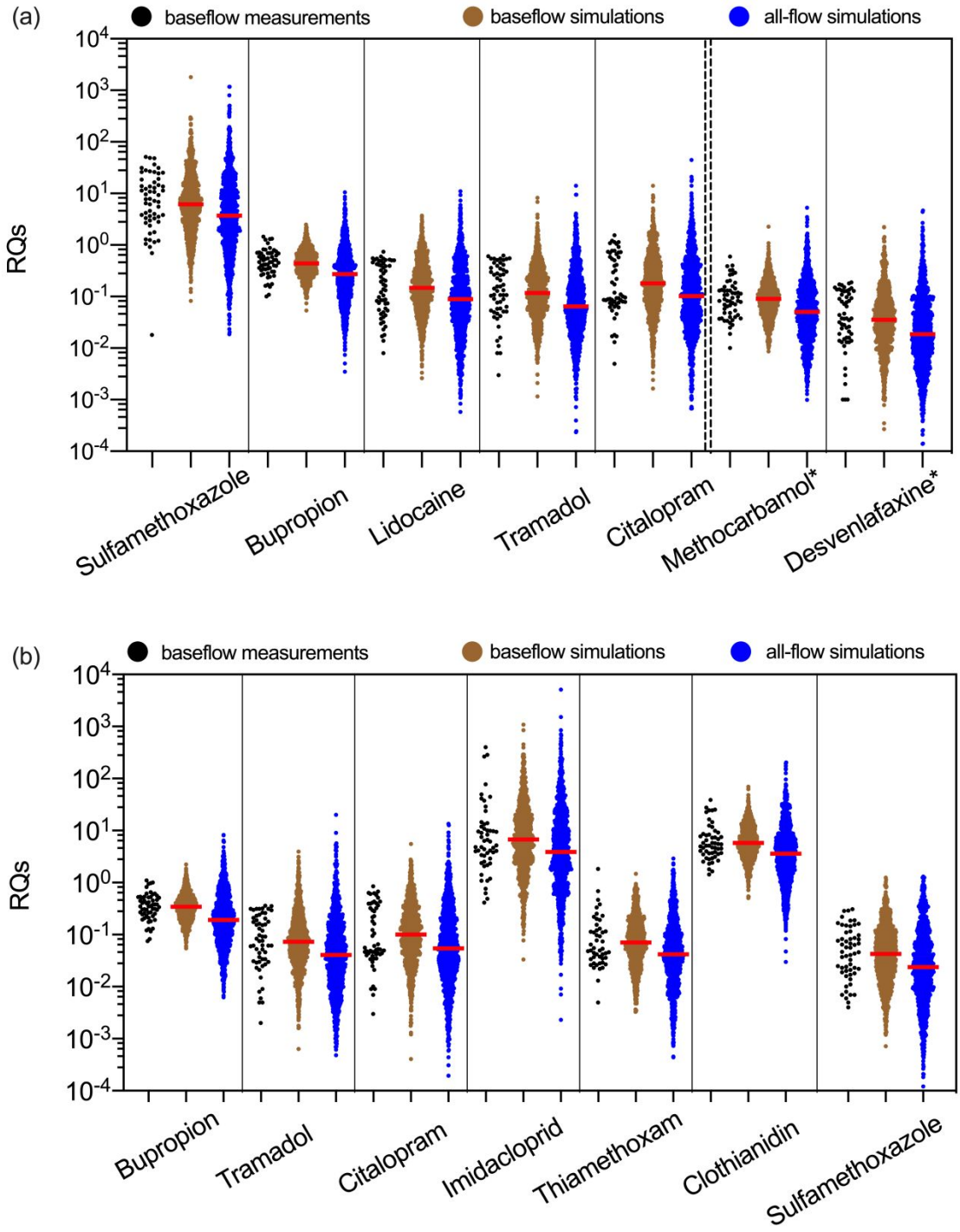


Figure 4: Measured and simulated risk quotients (RQs) of acute effect related to stochastic risk modeling. Measured values occurred under baseflow conditions, whereas the simulated conditions were generated via Monte Carlo simulations for baseflow and all flows during the two-year sampling period (flows determined at site DS2 using the USGS flow gage). Red solid lines represent median values for each simulated data set. Compounds were selected for stochastic risk simulation when the 75th percentile of the total measured RQs under baseflow conditions exceeded the lowest problematic risk level (i.e., RQ=0.1) for at least one of the three different aquatic species types (i.e., algae, invertebrates, fish). (a) RQ comparisons of CECs for algae and fish. (b) RQ comparisons of CECs for invertebrates.

* indicates RQs for fish. Only two compounds methocarbamol and desvenlafaxine exhibited medium or higher risks to fish.

For the majority of compounds, the measured RQs and simulated RQs under baseflow conditions exhibited similar distributions, demonstrating the robustness of the stochastic modeling approach (Figure 4, S.7). When comparing the RQ distribution and median values under baseflow conditions and all-flow conditions, RQs under all-flow conditions had a broader distribution (12–204% broader) with a decreased median RQ (Figure 4) which was expected due to dilution with non-effluent water as well as a wider flow distribution that encompassed a broader range of hydrologic conditions. Due to the relatively limited data available and the fact that we aggregated all concentrations from within the reach (i.e., all sites pooled together), some compound RQs in the simulation are likely quite accurate—while others may be less accurate. For example, citalopram is a rapidly-attenuated compound that is mainly derived from the effluent,[38,43] thus the measured RQs at DS1 are significantly higher than RQs at DS2 (roughly 9-fold; $p<0.0001$; Figure 4). In contrast, imidacloprid and sulfamethoxazole, are highly soluble compounds that both persist in the stream and are found at similar RQs between sites DS1 and DS2 ($p>0.05$; Figure S.8) and thus spatial differences within the reach are less important. For compounds that substantially changed concentrations along the reach, differences in concentration along the reach used as model inputs are only expressed as a broader input distribution to the model (due to the single segment assumption) and thus result in decreased overall accuracy. For example, because citalopram is rapidly attenuated via sorption within the reach, this assumption would systematically underestimate actual risk closer to the WWTP outfall and underestimate risk farther downstream while exhibiting greater overall uncertainly in the model results. We excluded atrazine from this model, due to the fact that atrazine is highest in US1 and is diluted by the treated effluent rather than derived from the treated effluent.

446

447

448 **3.2 Attenuation modeling in the stream and risks to a drinking water source**

449 Attenuation modeling predicted in-stream transport dynamics of effluent-derived CECs
450 (i.e., 14 pharmaceuticals), and demonstrated that the majority pharmaceuticals (13 out of 14)
451 persisted along the stream reach (median attenuation rate constant $k<0.1$ h^{-1}) and entered the
452 Iowa River at elevated concentrations. First, we used different measured field data (collected/
453 analyzed by UIowa during Year 1 of the study) to validate the simulation model calibration, and
454 to demonstrate that the attenuation behaviors of pharmaceuticals can be well-predicted by the
455 attenuation model during baseflow conditions (i.e., the model was externally validated with
456 additional field data, Figure S.10) and match our prior results probing mechanistic fates.[43] For
457 example, both measured results and simulation results indicate that citalopram was substantially
458 attenuated (>80%) along the 5.1 km stream reach, while for venlafaxine only moderate
459 attenuation (~50%) occurred along the stream reach. Citalopram and venlafaxine were selected
460 to represent rapidly-attenuated and moderately-attenuated compounds in the stream reach,
461 respectively.

462 Compared to some of the pesticides and industrial chemicals, pharmaceuticals were likely
463 exclusively derived from the WWTP discharge, making them more suitable to fit in the first-
464 order kinetics assumptions in the stream. The attenuation rate constants (k) of 14
465 pharmaceuticals determined were compound-specific.[42] Citalopram exhibited rapid attenuation
466 ($k=0.2187\pm0.0172$ h^{-1}, median± standard deviation,) compared to other pharmaceuticals (Figure
467 S.9; Table S.16). The rapid attenuation for citalopram is likely due to sorption,[69,70] as we
468 demonstrated in recent research using Muddy Creek stream bed sediments.[43] Other
469 pharmaceuticals persisted along the stream reach with median k-values 4–22 fold lower (0.0098–

0.0554 h^{-1}; half-life 12–71 h) compared to citalopram (Figure S.9; Table S.16). In the present study, metformin and guanylurea had median k-values of 0.0399 ±0.0038 h^{-1} and 0.0172 ±0.0094 h^{-1}, respectively. This agrees with previously reported attenuation rate constants of metformin (0.0028–0.0162 h^{-1}) and guanylurea (0.0058–0.0263 h^{-1}).[71] Persistence of carbamazepine (median k-value: 0.0021–0.0074 h^{-1}) and desvenlafaxine (median k value: -0.0004–0.0077 h^{-1}) has been reported,[71,72] which is one order of magnitude lower than k measured in the present study (0.0291±0.0023 h^{-1} and 0.0348±0.0022 h^{-1}, respectively). In contrast, a mean k value of 0.17 h^{-1} was measured for carbamazepine from four rivers in Spain.[73] Based on the attenuation model, limited attenuation from the Effluent to site DS2 during baseflow conditions indicated a substantial amount of CECs (ranging 0–47% of the initial concentration in the wastewater effluent) are constantly entering the Iowa River year-round, posing potential risks to aquatic biota throughout the Muddy Creek study reach and to the downstream drinking water source (Figure 5, S.12). Although dilution by a larger receiving water can substantially lower the concentrations when Muddy Creek enters the Iowa River, the continuous chemical input from the wastewater outfall to Muddy Creek still poses potential risks due to long-term consistent inputs, along with the existing complex chemical mixtures in the Iowa River.[74] For example, it is likely that elevated concentrations of pesticides are already present in the Iowa River.[74] Thus, the dilution effects by the Iowa River to in-situ biota under real-world conditions may not be as substantial as predicted due to mixtures present in the receiving water. Nevertheless, this modeling approach can be a useful prediction tool to help us understand changing ecological exposure risk throughout the stream reach. Thus, conducting attenuation modeling at Muddy Creek as a representative study reach, can improve our understanding of the ecological impacts and/or potential human exposure to CEC mixtures in effluent-dominated systems.

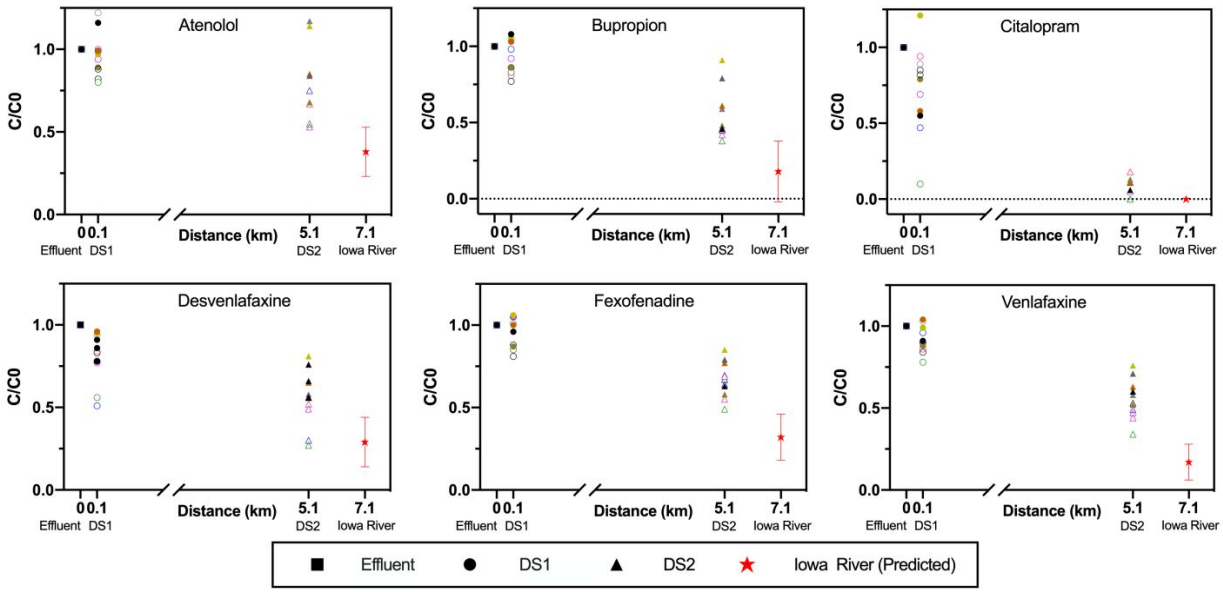


Figure 5: Measured (Effluent, DS1, DS2 in Muddy Creek, Iowa) and predicted concentrations (Iowa River) of selected pharmaceuticals in the effluent and along the stream reach (full figure in Supporting Information, Figure S.11). Distance "0" km represents the point at which the effluent mixes with the stream. The red star with standard error bar is the predicted concentration of the given chemical within Muddy Creek when Muddy Creek reaches the confluence with the Iowa River (i.e., before mixing with the Iowa River) based on the rate constant; it also corresponds the location of the red star in Fig. 1. Other data points at a given location are individual sampling dates measured results during Year 1.[38] Different shapes represent corresponding sampling locations. Different colors represent individual sampling dates.

The predicted concentrations of CEC mixtures after joining the Iowa River, as well as the measured concentrations in the DWTP influent and effluent, were below the human health benchmark concentrations (HHBs; Table S.18), indicating minimal exposure risks to humans. Despite the high levels of CECs (i.e., measured up to ~5,000 ng/L at DS2) in the stream, the concentrations are predicted to substantially decrease after dilution in the Iowa River (i.e., 55-100% attenuation, Table S.16). Nevertheless, as a 'worst-case scenario', CECs such as neonicotinoids and metformin will not be removed by the (conventional coagulation-flocculation) drinking water treatment plant, and thus may be present in the finished drinking water.[10,15,75] Previous studies from our laboratory indeed were the first to report the presence of three neonicotinoids in finished drinking water and demonstrated their general persistence during conventional drinking water treatment processes.[10,15] In the present study, our one-time snapshot

sampling of raw and finished drinking water from the UIowa DWTP also demonstrated the potential impacts of CECs in Muddy Creek on drinking water treatment intakes. CEC residuals of 0.2–325 ng/L were measured in the raw and finished drinking water (Figure 6). Despite concentrations being below HHBs, these CECs could still have potential deleterious effects when considered with the suite of other contaminants (e.g., pesticides, disinfection byproducts) known to be present in drinking water from this DWTP.[76] This was a single sampling event and we cannot track the specific sources of the CECs detected because multiple sources contribute to such concentrations in the Iowa River. These exploratory results, however, suggest the potential for CECs in effluent-dominated streams to affect corresponding drinking water sources (i.e., *de facto* water reuse), consistent with established work.[1–5] Furthermore, groundwater recharge (due to wastewater effluent influx) could cause CECs to be transported along subsurface pathways into adjacent aquifers and could pose potential risks to groundwater sources.[77]

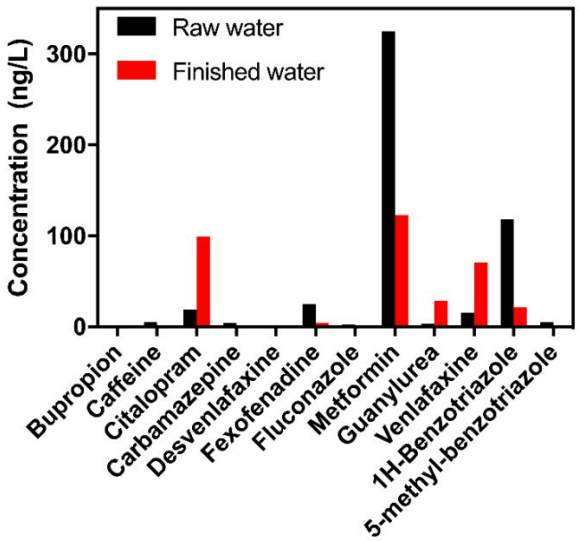


Figure 6: Select contaminants of emerging concern (CECs) measured in University of Iowa drinking water treatment plant (DWTP) for raw and finished drinking water during a one-time exploratory sampling event (May 12, 2018). Collected water samples followed the same procedures including sample process and analytical method with Muddy Creek water samples. "Raw water" is the screened raw water intake from the Iowa River, and the "finished drinking water" is the treated drinking water prior to the distribution system.

In the present work, we used two different modeling approaches, stochastic risk and attenuation transport modeling, to predict ecological risks of CECs in the stream under all-flow conditions and simulate the transport of effluent-derived CECs in the stream, respectively. The transport model provided attenuation rate constants that help us understand the attenuation behaviors of individual chemicals. Nevertheless, the transport model can only yield the attenuation percentage (C/C_0) rather than the actual environmental concentration, which is essential for RQ calculation and prediction. Stochastic risk modeling can help examine a wide range of biological endpoints under dynamic stream hydrologic conditions, and appears particularly useful to characterize changing risk dynamics of effluent-derived chemicals in an effluent-dominated stream. Furthermore, field work under baseflow conditions is inherently more feasible and thus incorporation of a stochastic modeling approach can serve as a useful risk prediction tool under variable input source and hydrologic conditions. However, the stochastic risk model yielded an average risk for the entire reach but could not account for changing risk due to in-stream attenuation. Thus, as described above, the approach we used worked well for compounds that persisted through the reach and were derived from the point source, but greater spatial resolution via accounting for in-stream attenuation is required for highly sorptive or labile compounds. Based on our recent work investigating attenuation mechanisms within the stream,[43] our approach would work well for most of the compounds studied (the single segment assumption does not approximate citrapram well). Integration of the two modelling approaches with additional data would develop a comprehensive risk assessment tool for effluent-dominated streams and could be the aim of future work; such a probabilistic transport model would provide greater accuracy and less uncertainty at a given site rather than the averaged approach for the entire risk taken here. Nevertheless, both the attenuation model and stochastic risk model in their

current forms provide fundamental insights on the fate of CEC mixtures and potential ecological risks of effluent-dominated streams to local ecosystems and to the drinking water source. In addition, current approaches to measured risk assessments in a stream often consider only limited site data / locations.[59,78] Thus, our proposed framework of integrating measured baseflow concentrations and gaged streamflow to probabilistically estimate risk may improve the practicality of estimating exposure risks to aquatic biota under variable conditions because baseflow conditions are inherently more practical to measure than runoff events (i.e., samples can be more-easily assured as representative). It is impractical (and too costly) to expect to address the needs for additional aquatic life risk assessment from effluent-derived chemical under variable flow conditions solely through the additional data acquisition. With enhanced understanding of temperate-region effluent-dominated streams, the long-term goal is to develop a comprehensive and easy-to-use prediction tool that can be applicable to other effluent-dominated streams and inform sustainable water resources decision making.

4. Conclusions

We assessed the ecological risks of different CECs in the stream under baseflow conditions and demonstrated that 11 out of 18 CECs (2 compounds did not have available toxicity data) may pose medium to high risks to local ecological systems (i.e., within the stream). Stochastic risk modeling shows a decreased risk of effluent-derived CECs due to dilution from stormflows; however, the overall decrease is risk exposure is relatively small and does not eliminate the risk. Indeed, this work highlights that mere dilution does not fully attenuate risk to aquatic biota in effluent dominated streams. We demonstrate that stochastic risk modeling is a useful approach to characterize exposure risk dynamics from effluent-derived chemicals under

581 variable flow conditions (i.e., dilution of effluent-derived chemicals by storm flows), and is
582 particularly useful when there is limited chemical data but adequate flow information (as is
583 common in continuously gaged stream)—this approach appears highly useful to characterize
584 effluent-dominated streams. Attenuation modeling predicted in-stream transport dynamics of
585 effluent-derived CECs (i.e., 14 pharmaceuticals), and demonstrated that the majority
586 pharmaceuticals (13 out of 14) persisted along the stream reach (median attenuation rate constant
587 $k < 0.1\ h^{-1}$) and entered the Iowa River at elevated concentrations. The predicted concentrations
588 of CEC mixtures after joining the Iowa River, as well as the measured concentrations in the raw
589 and finished drinking water within the DWTP, were below the human health benchmark
590 concentrations, indicating minimal risks to humans exposed to the target contaminants on an
591 individual basis. Nevertheless, these CECs could still have potential deleterious effects when
592 considered with mixtures of other contaminants (e.g., pesticides, disinfection byproducts) known
593 to be present in the raw and finished drinking water from this water source.
594
595
596 **SUPPLEMENTARY MATERIAL.** Additional method details, statistical analysis, quality
597 assurance / control, additional detailed data / results / analysis in figures and tables.
598
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601

CONFLICTS OF INTEREST.

There are no conflicts of interest to declare.

ACKNOWLEDGEMENT

This work was supported by grants from the U.S. Geological Survey Grant (2017 104(g) Grant Number G17AP00135 Project ID Project ID 2017IA502G), the National Science Foundation (CBET Environmental Engineering 1803197), Graduate Student Supplemental Research Competition Grant (15802100 (BR01)) from Iowa Water Center and University of Iowa Graduate School Fellowships from the University of Iowa and from programmatic support from the U.S. Geological Survey's Toxics Substances Hydrology Program. Danielle T. Webb was supported by the University of Iowa Center for Biocatalysis and Bioprocessing / NIH Predoctoral Training Program in Biotechnology (2 T32 GM008365) and the Alfred P. Sloan Foundation Sloan Center for Exemplary Mentoring. We thank undergraduate students Megan Powers and John Quin VI from the University of Iowa for sample collections, and Drew Lammers from the North Liberty Wastewater Treatment plant. We also thank Shannon Meppelink and Jessica Garett for their assistance collecting water quality samples and conducting instream hydrologic measurements. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government or the authors.

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