Assessing Human Health Risks and Prevalence of De Facto Reuse in Drinking Water

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Project Summary

This study, supported by the U.S. Environmental Protection Agency (EPA), aims to better understand the impact of de facto water reuse on public health and the environment. De facto reuse occurs when untreated or partially treated wastewater is unintentionally incorporated into drinking water sources. The study has two main objectives: first, to develop tools and models for water utilities to assess the effects of de facto reuse on water quality, and second, to evaluate the potential health risks by comparing water sources impacted by wastewater effluent to less-impacted sources, such as headwaters. The research will examine both pathogens and a range of chemical contaminants, including perfluoroalkyl and polyfluoroalkyl substances (PFA')s and dibutyl phthalate (DBP's), which are both classifiable carcinogens. Water samples will be collected from the Colorado River in Colorado and Utah at two sites in addition to natural control sites, and toxicity tests will be conducted using fathead minnows (*Pimephales promelas*). Statistical analyses will identify significant differences in water quality across sites and seasons. This study will provide important data for risk assessments, improving water quality standards, and shaping policies to manage water resources amid increasing scarcity and climate change. The findings will help create sustainable water reuse strategies, especially in areas affected by climate change, protecting both human and environmental health as water reuse grows. The research will also offer insights into the ecological impacts of de facto reuse, highlighting the need for water management practices that balance human needs with the health of aquatic ecosystems.

Challenge Definition and Introduction

De facto water reuse occurs when wastewater effluent is incidentally present in drinking water. This compares to other potable water reuse, where wastewater is treated with the intention of supplementing the water supply. De facto reuse is most noticeable in regions with high population density and low streamflow[1]. As water flows further from the headwaters, water will have higher rates of wastewater due to the cycle of cities' inputs and outputs into the river. Although this type of reuse can cause water quality issues with taste, odor, and pathogens, this input of water is still important to supplement flows. One study explored 80 USGS sites using a GIS system, finding that across these sites de facto reuse "increased from 3.6% under mean flow to 46% under low flow conditions"[2]. This study gives some insight into how de facto reuse will increase as more strain is put on the resource. For this reason, understanding de facto water reuse is important to mitigate possible issues that may arise. This issue is especially pressing in the American Southwest, where every drop of water is allocated and put to beneficial use. To keep water users happy, water reuse is a critical aspect of hydrology in the West.

Exposure to wastewater effluents can raise health concerns. The discharge from these plants are a sources of micropollutants in the environment, posing health concerns for both people and the ecosystem. These pollutants include endocrine disruptors, pharmaceuticals, personal care products, disinfection byproduct precursors, nutrients, pathogens, PFAS, pesticides[1,3]. Some of the health concerns for humans associated with these pollutants include—

but are not limited to — neurological issues, cancer, hormonal disruptions, gastrointestinal illnesses, methemoglobinemia (blue baby syndrome), and developmental issues. The ecosystem also faces health risks through reproductive failures, behavioral changes in aquatic animals, eutrophication, disease spreading in wildlife, and bioaccumulation. Figure 7-2 from the National Academies of Sciences, Engineering, and Medicine (2012) shows 3 scenarios with different treatment processes, one of which is de facto reuse. While this study acknowledges there could be errors in the magnitude of de facto considered in this case, the main finding still stands: when compared to other water treatment processes, de facto presents the highest risk (p.128). This raises concern because many of these pollutants are unregulated, resulting in them being overlooked during wastewater treatment[4]. A better understanding of the pathogens overlooked in this type of reuse can help develop a regulatory paradigm to protect all users of the water.

The idea of drinking and using former wastewater can be met with aversion from consumers because it may be perceived to be unclean, or it may taste different. Public opinions of this type of reuse are important to consider because they can influence implementation. One study of public acceptance took place across three cities — Atlanta, Philadelphia, and Phoenix— where water users were surveyed to measure attitudes regarding de facto wastewater reuse. This survey was coupled with estimations of actual extents of de facto wastewater reuse in the selected cities. From this survey, a key finding arose: knowledge and experience with this type of reuse are essential for acceptance of wastewater reuse[5]. For this reason, building a better understanding of the pathogens, which were listed earlier in the paper, in de facto reuse is important. As the National Academy of Engineering states, "achieving a high level of reliability, public health protection, and nationwide consistency in the regulation [would help build] public confidence" (2012, p.190). Collecting a plethora of data to bridge the gap between often confusing findings and transparent public communication can have a monumental impact when mitigating possible backlash associated with increasing water reuse.

The current body of research on de facto reuse reveals several gaps and weaknesses. Many of the models built to assess amounts of de facto reuse in the stream rely on stream data and data models to fill in gaps of data, especially through GIS programs. Existing stream data is very useful when it is accurate, but it can cause many issues when measurements are off or methods aren't consistent across research. Rice and Westerhoff provided an example of how using median streamflow or average streamflow can meaningfully change the results of studies. Using the median value can show higher levels of de facto reuse because averages can be skewed by extremes, resulting in possible underreporting of numbers for certain studies. As expressed in this article, "this shows the variability in calculating de facto reuse based upon the methodology selected" (2014, p.985). While both cases used sound reasoning to obtain results, the different methodologies employed can make meta-analysis less effective.

Further, there are often gaps in the location of gauges and periods of consistent data collection associated with the current methods used to study de facto reuse. Knowledge across smaller watersheds and streams where gauges aren't present is limited. This matters because if a wastewater treatment plant is in these areas, de facto water may account for a larger portion of the total water in the watershed. Consistent measurements are important because gaps in data require the use of different models to make information usable. In the case of the Yangtze River Basin in China, this study analyzes de facto reuse using the ArcGIS hydrological analysis tool, a tool used to model the flow of water. In this research, the hydrological dataset is limited and there is imprecise locational and design data about the treatment plants[1]. Using an analysis tool requires the study to operate under a set of assumptions. If any of these assumptions are violated, it can lead to unreliable conclusions. Furthermore, if the model does not accurately represent what real streamflow data would look like, it can introduce bias and reduce the validity of any conclusions drawn. Having better data and spatiotemporal knowledge can yield better knowledge of the extent of de facto reuse in a watershed and provide data useful for "estimating contaminant attenuation between effluent discharge and potable water intakes (e.g., residence time, water quality, depth)"[4]. This information is critical for improving risk assessments, informing regulatory decisions, and ensuring the safety of drinking water supplies in watersheds affected by de facto reuse.

While de facto water use becomes more prevalent in the western United States, there is still a lack of in-depth scientific research and understanding on the topic. This research aims to help lessen that gap and further understand the topic. De facto water use can help aid in the battle against water insecurity on a significant spatial and temporal scale, improve public health standards, and overall contribute to the broader body of knowledge of water resources in light of climate change.

Technical Merit

The proposed research will achieve the goal of informing the public and private sectors about the health and safety risks of consuming defacto water; thus giving them the opportunity to make informed decisions about water use. Informing the public sector will be accomplished through De Facto Water seminars hosted by local municipality councils, and the private sector will receive deliverables tailored to their operations. For example, a private water plant and distributor would receive the key aspects of our study along with applications they can use in their daily practice, while the public will be emphasizing education and dampening the application aspects. Due to water becoming more scarce in an increasingly arid climate, this research will be able to inform necessary discussions about water allocation in water-insecure communities.

Challenges within our research include the public disclosure of locations used for testing, which will be addressed through general area descriptions and volume of water without specifically specifying the testing site or community impacted to minimize widespread reactions. Using streamlined assumptions and subjects within our study would aid the misconceptions commonly generated after vague data sources are identified. Using the funding from the Environmental Protection Agency (EPA) would advocate for the integrity of the research due to the interest of the agency providing information concerning public health. Due to this data being the first of its kind, we would highly encourage retesting and advocate for further examination of de facto water reuse in a controlled environment.

Relationship of Challenge to Sustainability

Relating to the United Nations' Sustainable Development Goal (SDG) of 'ensuring clean water and sanitation for all', this research aims to provide insight into the future of water use in its functionality as a pollutable resource. Water insecurity is a growing challenge in the face of increasing populations, especially in the United States West of the Mississippi River. Policy concerning the use and right to water in this area prioritizes upstream users in water allocation on a 'first in time, first in right' basis. For example, Nevada relies heavily on the Colorado River for the urban water supply, but its water access depends on the usage of upriver states like Colorado and Utah. During droughts, this policy in combination with upstream withdrawals can reduce the flow of water reaching Nevada, increasing the risk for water scarcity even with existing interstate compacts in place.

De facto water reuse brings the opportunity to enhance resiliency to challenges like water insecurity in these regions. While not always formally regulated, water reuse becomes a critical supplemental water source in times of scarcity. By integrating de facto water reuse into multi-level policy and long-term planning, states can help minimize the impacts of drought and adapt to uncertain water allocation.

Measurable Results

We propose a study to evaluate public health risks and water quality impacts associated with de facto wastewater reuse in different spatial and temporal scales. On account of the limited availability of updated data, the development and implementation of potable reuse systems vary significantly across geographic regions. Evaluating states that currently employ potable reuse alongside those that do not enable large-scale comparative analyses of the social, environmental, and sustainability-related implications associated with this practice. Colorado represents a leading example in the implementation of water reuse practices, driven in part by its arid climate, whereas Utah serves as a contrasting case with limited adoption of such strategies. Furthermore, to provide robust evidence of the effects—or absence thereof—of de facto water, natural control water bodies are utilized, particularly to enhance the standardization and reliability of the fisheries bioassay.

In Colorado, the controlled monitoring location will be established near the headwaters of the Colorado River flowing from Rocky Mountain National Park, where the primary water source is snowmelt. Two additional sites will be selected immediately downstream of wastewater treatment plant discharge points and upstream of drinking water treatment facilities, allowing for direct comparisons between relatively pristine and impacted waters. These values can then be compared to treated water that runs through the tap. This would require a partnership with a municipality interested in exploring water reuse, for example, Castle Rock, CO. Another key location to consider is Utah, which lies downstream along the Colorado River. Despite its arid western climate, the state has comparatively minimal implementation of water reuse practices. The lack of implementation in this region is largely because of public opinion, policy constraints, and most prudent the concern for impacts to the Great Salt Lakes.

In addition to assessing spatial variation, this study aims to identify temporal patterns in de facto water reuse, as reuse rates have been shown to fluctuate seasonally in response to climatic variability. Therefore, a collection of samples should be taken during a period of peak flow when the river is at its highest streamflow and stage, then again in late summer when flows are low. It should be noted that we do not want a large span where flow is absent through areas of the river where we are studying. Water samples collected from Colorado and Utah along the Colorado River will be analyzed to assess water quality using both chemical and toxicological methods. Specifically, this research would monitor quality by analyzing disinfection by-products (DBPs), polyfluoroalkyl substances (PFAS), nitrogen, phosphorus, and salt. To measure DBPs, we would use EPA Method 524.2: Measurement of Purgeable Organic Compounds in Water by Capillary Column Gas Chromatography/Mass Spectrometry. This method applies to a wide range of organic compounds. This method works by extracting volatile organic compounds with inert gas. It is then desorbed into a gas chromatograph mass-spectrometer for separation, identification, and quantification using internal and calibration standards[6]. In order to conserve cost, we will be sending out samples to labs for analysis. Samples would be collected from the water, then stabilized and put into a cooler until sent to the lab. Measuring various parameters such as nitrogen, salt, dissolved solids, and ore can be accomplished with a sonde. This is a probe that is inserted into the water. This would require people to be on site to measure these parameters.

To measure PFAS, we will use EPA Method 537.1 revision 2: Determination of selected per- and polyfluorinated alkyl substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography / Tandem Mass Spectrometry. This measurement technique is done with a water sample that is run through a cartridge to collect target compounds. These compounds are concentrated and measured by liquid chromatography/tandem mass spectrometry. Compounds are identified by comparing them to known standards, and their amounts are measured using internal standards[7]. Due to the high cost of a mass spectrometer on top of all the lab equipment, we will be sending out samples to labs for analysis. Samples would be collected from the water, then stabilized and put into a cooler until sent to the lab.

Using the data gathered from these samples, we would analyze various parameters:

- Correlating effluent contribution percentage, using known discharge volumes and flow data, to contaminant concentrations at intakes and in treated water.
- Estimate the relative risk
- Discover if any negative trends arise from contaminant concentrations after being treated by the water treatment facility to see if treatment plants are equipped to treat contaminants associated with increased water reuse.

- Compare the risk of the reservoir compared to the river water, both supplemented with de facto water

In addition to water quality assessments, ecological bioassays and toxicological tests will be conducted to facilitate extrapolation to natural environments, particularly given that the control samples consist of natural water bodies. Water samples from each site will be utilized to assess acute toxicity using *Pimephales promelas* (fathead minnow) as the test organism, with mortality serving as the primary endpoint. Fathead minnow is the optimum test organism for this study as they are easily cultured and readily available as well as common in toxicity studies therefore allowing for standardized comparisons. The exposure protocol will consist of a 14-day trial, with a 96-hour primary exposure period followed by a 10-day observation phase to monitor sublethal and lethal effects. Five fish will be exposed to water from each site in 5-gallon tanks for the duration of the trial along with control sites. Three replicate tanks will be used for each site, yielding a total of 12 experimental tanks (Figure 2). Each trial will be repeated three times to ensure reliability and statistical power. Data will be subjected to a two-way analysis of variance (ANOVA) to evaluate the effects of site location and exposure on fish mortality rates. In the case of significant differences, a Tukey post-hoc test will be employed to further delineate which sites exhibit statistically significant disparities in mortality. This experimental design will provide a robust framework for evaluating spatial and temporal variations in water quality and their potential impacts on aquatic organisms.

Broader Impacts

This study aims to advance the understanding of de facto water reuse by providing critical insights into its prevalence, potential risks, and ecological consequences. As the reliance on de facto reuse—where treated wastewater inadvertently enters drinking water sources—continues to increase, particularly in regions experiencing heightened water scarcity and climatic variability, this research seeks to refine the existing body of knowledge. Previous studies have shown that the proportion of de facto water in drinking supplies has grown significantly over time, with wastewater effluent accounting for a greater share of river flow under low-flow conditions. Our research builds upon these findings by developing comprehensive baseline data that captures both the spatial and temporal dimensions of de facto water use. This expanded dataset will enhance public health risk assessments, contribute to the formulation of evidence-based policies, and support the continued evolution of regulatory frameworks, similar to the progressive water reuse strategies already adopted in states such as Colorado.

In addition to assessing the potential human health risks associated with de facto reuse, this study will evaluate its environmental impacts, particularly on aquatic ecosystems. The incorporation of ecological bioassays, including the use of fish as bioindicators, allows for a holistic approach that addresses the health of both human and natural systems. Given the potential for wastewater contaminants, such as pharmaceuticals and endocrine disruptors, to affect aquatic organisms, the findings of this research may call for updated water quality standards that consider both human and ecological health. By evaluating the safety and sustainability of de facto water as a resource, the study will inform future water reuse strategies, especially in water-stressed regions, and will underscore the need for integrated water management practices that address both public health and ecological integrity. Ultimately, the outcomes of this study will contribute to the development of robust, science-based guidelines for managing and regulating de facto water reuse in a manner that supports the long-term health and sustainability of both human populations and the ecosystems they depend on.

Project Timeline

From start to finish, the goal for this project is to be completed in three years. This includes literature review, data collection and analysis, and writing up the final paper to prepare it for submission. Several of these stages will be occurring simultaneously. Also, the duration of each stage has a rough estimate of completion due to factors such as scheduling, funding, and travel.

Stage	Activity	Estimated duration	Deliverable
Literature review	Search, capture and synthesise relevant literature on de facto water use in the western United States	~3-6 weeks	Notes and other output from the review process
	Look at policies showing how public human health research translates back into the community		
Data collection	Finalize sampling plan	~2-3 years	Sampling plan
	Carry out data collection		Raw data
	Write up data collection		Draft data collection section for final report
Data analysis	Prepare data for	~5-6 months	Data ready for analysis

Stage	Activity	Estimated duration	Deliverable
	analysis		
	Analyse data		Notes and other output from analysis
	Draw conclusions/ recommendations		Draft data analysis and findings section final report
Writing up	Final draft of application	~2-6 months	Final draft
	Review draft		Notes on feedback
	Final editing		Final application
	Submit		

Budget

	Items	Year 1	Year 2	Year 3	
I. Personnel					
	Postdoc	$50,000.00	$50,000.00	$50,000.00	
	Postdoc Fringe (Yr 1:14.1% Yr 2&3: 28.7%)	$7,050.00	$14,350.00	$14,350.00	
	3 Undergraduate (No summer pay)	$15,000.00	$15,000.00	$15,000.00	
	Advisor	$110,000.00	$110,000.00	$110,000.00	
	Total Personell	$182,050.00	$189,350.00	$189,350.00	
II. Equipment					
	Tanks	$900.00			
	Aerators	$980.00			
	Sample Containers	$200.00			
	Syringes	$197.00			
	Silica gel	$232.00			
	sodium thiosulfate	$80.00			
	cooler	$300.00			
	ProDSS Multiparameter Digital Water Quality Meter	$4,000.00			
III. Supplies					
	Fish feed	$468.00	$468.00	$468.00	
V.Samples					
	Mass spectrometry samples ($5 per sample)	$240.00	$240.00	$240.00	
	Liquid Chromatography samples ($5 per sample)	$240.00	$240.00	$240.00	
	Shipping cost	$40.00	$40.00	$40.00	
	Per- and Polyfluoroalkyl Substances (PFAS) EPA 537.1 Rev 2.0	$90,000.00	$90,000.00	$90,000.00	
	EPA Method 524.2	$33,333.00	$33,333.00	$33,333.00	
VI. Travel					
	Gas for Field Sampling	$600.00	$600.00	$600.00	
	Per Diem for personnel	$15.50	$15.50	$15.50	
	Scientific Meetings	$2,000.00	$2,000.00	$2,000.00	
VIII. Indirect Cost (53.5%)		$187,027.17	$194,367.37	$194,367.37	
VIII. Total Cost					Total overall:
		$684,952.67	$700,003.87	$700,003.87	$2,084,960.41

Budget Justification

I. Personnel:

Funding is requested to support one postdoctoral researcher ($50,000/year), whose role will include experimental design, data analysis, and mentoring undergraduate assistants. Fringe benefits are calculated at 14.1% for Year 1 ($7,050) and 28.7% for Years 2 and 3 ($14,350 each year), based on institutional rates. Three undergraduate research assistants will assist with data collection, sample processing, and fish husbandry throughout the academic year (no summer pay), with a total annual cost of $15,000. The PI will dedicate significant time to project oversight, experimental planning, and reporting. The advisor salary is listed at $110,000 annually as part of institutional support, not charged directly to the grant.

II. Equipment:
Initial-year equipment purchases include fish tanks ($900), aerators ($980), sample containers ($200), syringes ($197), silica gel for sample preservation ($232), sodium thiosulfate for dechlorination ($80), and a cooler for sample transport ($300). A ProDSS multiparameter water quality meter ($4,000) is necessary for field and lab measurements of key water quality parameters.

III. Supplies:
Fish feed is required to sustain study animals and is estimated at $468 annually.

V. Samples:
Analytical costs include annual mass spectrometry and liquid chromatography analyses (48 samples/year each, $5/sample, totaling $240/year each). Shipping of biological and chemical samples is estimated at $40/year. PFAS analysis (EPA Method 537.1 Rev 2.0) is a central part of the study and costs $90,000/year. Additional chemical analysis using EPA Method 524.2 will cost $33,333 annually.

VI. Travel:
Travel includes field sampling trips, with gas estimated at $600/year. A minimal per diem ($15.50/year) supports occasional long sampling days. Funds for conference attendance and presentation of results at scientific meetings are budgeted at $2,000/year.

VIII. Indirect Costs:
Indirect costs are calculated at the institution's federally negotiated rate of 53.5%, totaling $187,027.17 in Year 1, $194,367.37 in Years 2 and 3.

Total Project Cost:
The total cost over three years is $2,084,960.41, with annual budgets of $684,952.67 (Year 1), $700,003.87 (Year 2), and $700,003.87 (Year 3).

Figures

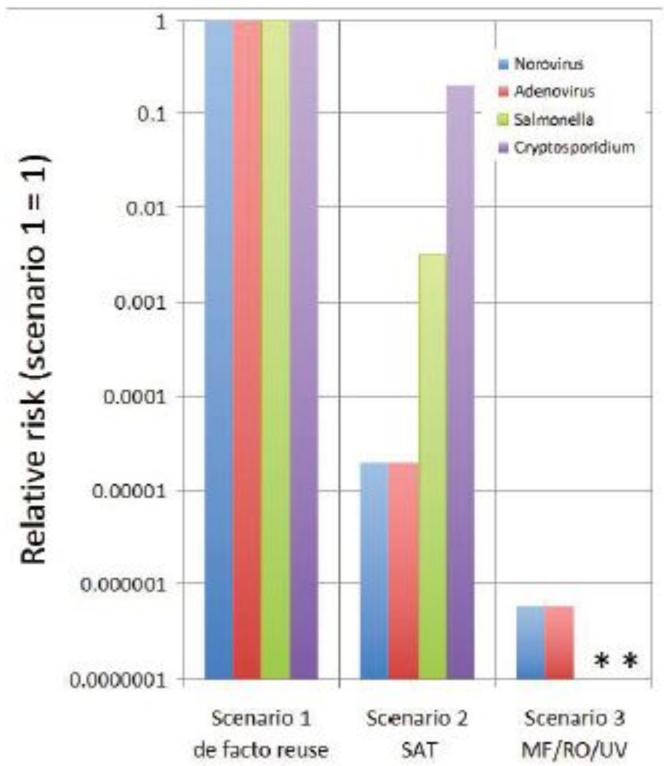

Figure 7-2.Relative risk of illness (gastroenteritis) to persons drinking water from each of the reuse scenarios relative to de facto reuse (Scenario 1). The smaller the number, the lower the relative risk of the reuse applications for each organism. *The risks for Salmonella and Cryptosporidium in Scenario 3 were below the limits that could be assessed by the model. From National Academies of Sciences, Engineering, and Medicine. 2012. Water Reuse: Potential for Expanding the Nation's Water Supply Through Reuse of Municipal Wastewater. Washington, DC: The National Academies Press. https://doi.org/10.17226/13303.

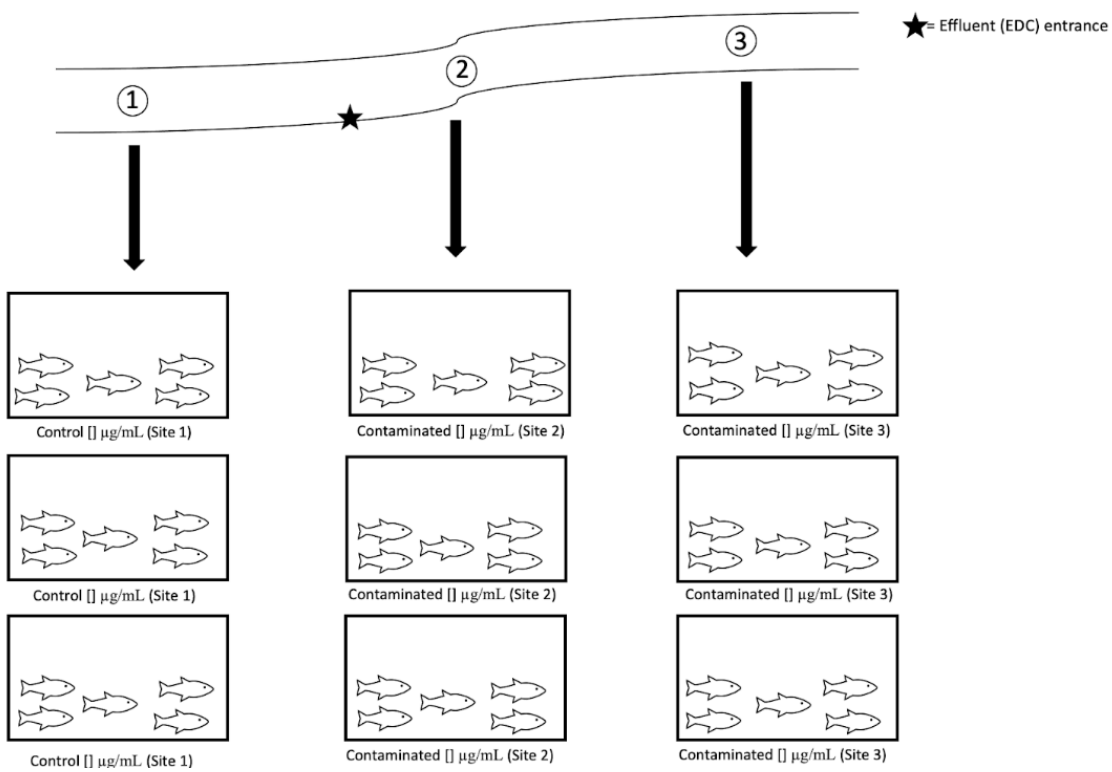

Figure 2. Field concentrations from Colorado River (Colorado) sites 1, 2, and 3 were measured to inform laboratory acute and lethal endpoints. Site 1 represents control conditions, while sites 2 and 3 are situated post- water reuse facility. These concentrations were replicated in 5-gallon tank's housing five fathead minnow (*Pimephales promelas*), with three replicates for each concentration. Control and contamination concentrations, indicated by [], are expressed in µg/mL.

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