Department of Pesticide Regulation
Environmental Monitoring Branch
Surface Water Protection Program

1001 I Street
Sacramento, CA 95812

STUDY 322: Monitoring Pesticides in Wastewater Influent and Effluent (2024)

John Wheeler
October 2023

1.0 INTRODUCTION

The occurrence of pesticides in treated wastewater effluent at concentrations that exceed aquatic toxicity thresholds has been documented in wastewater treatment plants (WWTPs) in California (Sutton et al., 2019). Down-the-drain pesticide transport may result from direct application to drains or indirect transport from other indoor or outdoor applications (Xie et al., 2021). Residential indoor sources, such as foggers/sprays (Dery et al. 2022), topical applications to domestic pets, or pesticide treated textiles may enter the waste stream through activities including washing, bathing, or laundry. For example, the application of topical flea and tick treatment products to dogs, and subsequent bathing, is a direct source of fipronil loading to municipal wastewater systems (Teerlink et al., 2017). Additionally, multiple pesticide active ingredients (AIs) used in pet products (fipronil, permethrin, and imidacloprid) have been detected in sub-sewershed laterals (i.e., pipes that connect a structure to a municipal main sewer line) serving dog grooming businesses (Budd et al., 2023). Pesticides that are applied outdoors may be transferred to a person's clothing or shoes, which may ultimately be transported down-the-drain through cleaning activities.

Pyrethroids have been detected in treated wastewater effluent of California WWTPs at concentrations that exhibited sub-lethal effects for sensitive invertebrates (Weston et al., 2013). A recent survey of eight WWTPs in the San Francisco Bay Area detected fipronil and imidacloprid in both influent and effluent samples, with little observed removal regardless of level of treatment (e.g., secondary, tertiary) (Sadaria et al., 2017). These regional stand-alone studies indicate the potential for pesticides within the sewershed to pass through WWTPs and discharge to surface water at concentrations that exceed toxicity thresholds such as the United States Environmental Protection Agency (USEPA) chronic aquatic life benchmarks. Additionally, inputs from wastewater outfalls into aquatic environments are usually constant, long-term, and uninterrupted. In order to understand the potential risk posed by pesticides in wastewater effluent to California aquatic habitats, a more comprehensive analysis of representative pesticide loading within the sewershed and subsequent discharge to surface water is warranted.

Similar to observations made for urban and agricultural runoff, it is feasible that variances in regional pest pressures could result in differences in pesticide use, resulting in subsequent

regional differences in composition of pesticides entering the wastestream (Ensminger et al., 2013). WWTPs have a wide range of treatment capabilities before discharging effluent. The majority of facilities are equipped with at least secondary treatment, and many have additional tertiary processes. Final effluent may be additionally treated with a disinfectant such as UV radiation or chlorine prior to leaving the facility. Available studies suggest even with the highest level of treatment, certain pesticides are present in effluent at concentrations that exceed toxicity thresholds (Sadaria et al., 2017; Budd et al., 2023). There is currently little understanding of the spatial and temporal variation of pesticides entering individual sewersheds. Further, there is limited data characterizing the potential for pesticide transformation and removal during various wastewater treatment processes.

The monitoring effort described herein builds on the California Department of Pesticide Regulation's (CDPR's) initial efforts to establish a long-term monitoring network for pesticides in wastewater in order to characterize the composition and magnitude of pesticides entering the wastestream. Information gained from this effort will allow assessment of differences in concentrations due to region, surrounding land use, and facility treatment level. Additionally, collected data may help elucidate pesticide transformation and removal processes that may occur within the wastewater treatment system. This protocol will be updated on an annual basis. Subsequent year protocols may incorporate additional study objectives.

2.0 OBJECTIVES

The overall goal of this project is to assess pesticide concentrations found in wastewater influent and effluent in California. Specific objectives include: (1) Determine the presence and concentrations of selected pesticides in wastewater influent and effluent; (2) Evaluate regional and seasonal variability in wastewater pesticide loading to WWTPs; (3) Evaluate the influence of sewershed characteristics (e.g., population, contributing land use) on relative pesticide loading; (4) Collect data to help elucidate pesticide transformation and removal processes within wastewater treatment systems.

3.0 PERSONNEL

The study will be conducted by staff from CDPR's Environmental Monitoring Branch, Surface Water Protection Program (SWPP), under the general direction of Dr. Anson Main, Ph.D., Environmental Program Manager. Key personnel are listed below:

Project Leader: John Wheeler
Reviewing Scientist: Robert Budd, Ph.D.
Statistician: Xuyang Zhang, Ph.D.
Laboratory Partner: Department of Toxic Substances Control (DTSC), Environmental Chemistry Laboratory - Pasadena (Contract #23-C0004)
Collaborators: Wastewater Treatment Plants throughout California
Please direct questions regarding this study to John Wheeler, Senior Environmental Scientist (Specialist), by email at John.Wheeler@cdpr.ca.gov (preferred contact method) or by phone at (916) 445-4026.

4.0 STUDY PLAN

4.1 Site Selection.

Monitoring sites will be chosen based on their ability to address study objectives. Volunteer WWTPs throughout California will be identified through direct contact with plant management and technical staff. Participating WWTPs will span a wide range of comparative parameters, including geographic region, size (measured in gallons treated per day), treatment capability (secondary or tertiary), final treatment (disinfectant), surrounding land use patterns (e.g., urban, agricultural), and point of discharge (freshwater or marine). Participating plant information is summarized in Table 2. Volunteer WWTPs will be asked to commit to participating for a period of 1 to 2 years at a time; however, details will be determined on a plant-by-plant basis. The goal is to obtain commitments from up to 30 WWTPs. Additional WWTPs may be included as participation in the program increases.

4.2 Pesticides for Analysis.

Target analytes were identified through a variety of methods. For example, SWPP staff conducted retail store surveys to identify pesticide products and associated AIs available directly to the consumer with potential for down-the-drain transport including pet products (Vander Werf et al., 2015; Budd & Petters, 2018). Additional analytes were prioritized through an evaluation of product labels to identify active products with registered indoor uses with the potential to enter the waste stream. Lastly, pesticides not identified in the preliminary list of target analytes that have been detected in wastewater effluent in previous research efforts (Sutton et al., 2019) were also added to the current analyte list. Analytical methods were developed during a previous collaborative project (Contract #18-C0159) with UC Davis. The DTSC Environmental Chemistry Laboratory adjusted methods where necessary to account for laboratory specific conditions. The list of target analytes for the current project is presented in Table 3.

Surface Water staff continue to identify AIs used in products with potential for down-the-drain transport. Moving forward, SWPP staff will work with DTSC's Environmental Chemistry Laboratory to develop analytical methods for additional analytes of interest, when feasible.

4.3 Sample Collection.

All influent and effluent samples will be collected and shipped by the participating WWTPs. Sampling bottles, shipping coolers, and prepaid shipping labels will be provided by CDPR. Collection methods will follow methods consistent with individual plant collection protocols using 24-hour composite (either flow-weighted or time-weighted) for influent and effluent samples, when available. If composite sampling is not feasible, "grab" samples will be accepted. Influent samples will be collected after the preliminary filtration and before primary treatment. Effluent samples will be collected at the end stage of physical treatment, but may be taken prior to the disinfection step. For each sampling event, participating plants will be asked to complete a chain-of-custody (COC) record provided by CDPR, which will include space to record details such as sampling date/time and collection method.

Samples will be collected in 125 ml and 250 ml amber glass bottles provided by CDPR. Specifically, most WWTPs will be asked to collect 500 ml of influent (4 x 125 ml bottles) and 1,500 ml of effluent (6 x 250 ml bottles) per monitoring event (the different volumes of influent and effluent are necessary due to differences in the analytical methods used for these two sample types). One WWTP per sampling event may be asked to collect an additional 125 ml influent sample for laboratory quality control purposes.

Influent and effluent samples will be shipped on ice within 24 hours of collection using CDPR-provided coolers and prepaid shipping labels to DTSC's Environmental Chemistry Laboratory in Pasadena for pesticide analysis. Additionally, effluent samples will be analyzed by SWPP staff for total organic carbon (TOC), dissolved organic carbon (DOC), and total suspended solids (TSS). Additional water quality parameters and details specific to collected sample (i.e., daily flow data) may be provided by individual WWTPs.

Influent and effluent sampling will be conducted up to three times per year at each of the participating WWTPs (Table 4). CDPR may attempt to coordinate the timing of sample collection to ensure all samples within the same sampling event are collected within a similar time frame, while providing flexibility to plants that may have scheduling constraints of their own. In order to minimize sample hold times, sampling during the beginning of the week is generally preferred (i.e., Monday through Thursday), so that samples can be shipped overnight to DTSC's Environmental Chemistry Laboratory. Sampling events will be spaced throughout the year to account for possible seasonal variation in pesticide concentrations. CDPR will make note of any sampling events that occur during a period of heavy rainfall, because this information may help to interpret the data obtained from the sampling event.

4.4 Changes from Past Protocols

This project is a continuation of past monitoring efforts. Here, SWPP staff have made several changes from the November 2022 monitoring protocol, as shown in Table 1, below.

Table 1 - Changes made to this monitoring protocol, compared to the November 2022 version.

Section of Document	Description of Change(s) Made
2.0 Objectives	Added new objective: elucidate pesticide transformation and removal processes.
6.0 Data Analysis and Reporting	Added the "NADA2" package to the list of R packages used. Removed Minitab from the list of data analysis software used.
7.0 Timeline	Updated the timeline to reflect the current project year: FY23-24.
Table 2	Revised the facility counts and plant capacities in the table to reflect current WWTP participants.

5.0 CHEMICAL ANALYSIS

Samples will be analyzed for pesticides by DTSC's Environmental Chemistry Laboratory in Pasadena according to the methods developed under CDPR Contracts 20-C0060 and 23-C0004. Quality control procedures include the use of a method blank, laboratory control sample,

laboratory control sample duplicate, matrix spike, matrix spike duplicate and sample duplicate with each batch of samples analyzed. Detailed descriptions of the analytical methods are available on the SWPP Analytical Methods webpage: https://www.cdpr.ca.gov/docs/emon/pubs/em_methd_main.htm

The TOC and DOC in samples will be analyzed by SWPP staff at CDPR's Bradshaw Regional Office using a Vario TOC Cube TOC/TNb Analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany) based on the protocol by Ensminger (2013a). Before analysis of every sample set, lab blanks and calibration standards will be run to ensure the quality of the TOC and DOC data.

All TSS in samples will be analyzed by SWPP staff by filtering the water samples using pre-weighed glass microfiber filters (Whatman GF/F 1825-090, 0.7 micron), drying them thoroughly, weighing them on an analytical balance, and calculating the mass of sediment retained on the filter (Ensminger, 2013b). For quality control, a 1-L sample of deionized (DI) water will be filtered with each batch of samples.

6.0 DATA ANALYSIS AND REPORTING

6.1 Data Analysis.

Environmental pesticide monitoring data are typically heavily skewed and contain a number of results that are below reporting limits (RLs). Statistical analysis of datasets with multiple RLs may violate the normality and equal-variance assumptions of parametric procedures such as analysis of variance (ANOVA) and *t*-tests. In order to appropriately address the characteristics of the sample data, a more generic and distribution-free approach, such as non-parametric statistics, will be used in this study. The application of non-parametric procedures is key to accurately interpreting skewed and censored environmental data (Helsel, 2012). SWPP staff will primarily reference Helsel (2012) as a general guideline for data analysis of this study. The data will be analyzed by using the R statistical program (R Core Team, 2014), specifically the "Nondetects and Data Analysis" for environmental data (NADA and NADA2) packages for R. In addition, SWPP staff will use non-parametric methods for the analysis of concentration differences among different factors and trends over time.

Based on the study objectives, preliminary analysis, and data availability, SWPP staff propose the following statistical procedures for data analysis (Table 5): Explanatory data analysis will be performed to summarize the characteristics of the sample data. Plots such as boxplots, histograms, probability plots, and empirical distribution functions will be produced to explore any potential patterns implied by the data. Hypothesis tests will be conducted to compare the concentration between groups of interest. Non-parametric procedures will be used to compute the statistics for hypothesis testing. Data with multiple RLs will be censored at the highest RL before proceeding, if the test procedure allows only one RL.

6.2 Data Reporting.

CDPR staff will provide each participating facility with a copy of their facility's pesticide analytical data, upon request.

Collected data will be summarized in annual data reports and may be presented in peer-reviewed journal articles. In addition, the data collected from this project may be used to develop or calibrate a down-the-drain pesticide model.

In all public-facing materials (e.g., data reports, peer-reviewed journal articles), CDPR will not associate final results with specific plant locations or identities without express written consent of the participating plant. Otherwise, all pesticide concentration data and results will be presented in an anonymized format. Participating plants will be granted the opportunity (minimum of 30 days) to review written reports or journal articles prior to publication.

7.0 TIMELINE

Field Sampling: January 2024 – December 2024
Chemical Analysis: January 2024 – March 2025
Summary Report: July 2025

Table 2 - Summary of participating WWTPs in monitoring study. Additional WWTPs may be added throughout the study to support study objectives. For some facilities, a portion of effluent is recycled while the remainder is discharged to a water body. In this table, facilities are classified based on the majority of the effluent volume (i.e., if >50% of the effluent is recycled, the facility would be classified as "Recycled" in this table).

Facility Treatment	Discharge Point	Number of Facilities	Plant Capacity (millions of gallons per day; MGD)
Secondary	Ocean/Bay	11	6.7 to 400
	Fresh Water	2	0.2 to 1.6
Tertiary	Ocean/Bay	2	8.5 to 39
	Fresh Water	9	9.9 to 392
	Recycled	2	12 to 15
Total		**26**	**0.2 to 400**

Table 3 - Pesticides to be monitored for in wastewater influent and effluent, with their respective reporting limits (RLs). Instrumentation: GC-QTOF = Gas chromatography with quadrupole time-of-flight mass spectrometry; LC-QQQ = Liquid chromatography with triple quadrupole mass spectrometry. Influent and effluent RLs are approximate, and are subject to change based on laboratory performance.

Pesticide	Instrumentation	Influent RL (ng/L)	Effluent RL (ng/L)
Bifenthrin	GC-QTOF	40	20
Bioallethrin	GC-QTOF	20	10
Chlorothalonil	GC-QTOF	20	10
Chlorpyrifos	GC-QTOF	20	10
Cyfluthrin	GC-QTOF	20	10
beta-Cyfluthrin	GC-QTOF	10	5
Cyhalothrin	GC-QTOF	40	20
gamma-Cyhalothrin	GC-QTOF	13	7
Cypermethrin	GC-QTOF	20	10
alpha-Cypermethrin	GC-QTOF	4	2
Cyphenothrin	GC-QTOF	100	50
Deltamethrin	GC-QTOF	100	50
Esfenvalerate	LC-QQQ	40	20
Etofenprox	LC-QQQ	20	10
Fenpropathrin	LC-QQQ	100	50
Fipronil	GC-QTOF	20	10
Fipronil amide	GC-QTOF	20	10
Fipronil desulfinyl	GC-QTOF	20	10
Fipronil desulfinyl amide	GC-QTOF	20	10
Fipronil sulfide	GC-QTOF	20	10
Fipronil sulfone	GC-QTOF	20	10
Imidacloprid	LC-QQQ	20	10
Novaluron	LC-QQQ	20	10
Permethrin	LC-QQQ	1,000	500
Phenothrin	GC-QTOF	1,000	500
Prallethrin	GC-QTOF	20	10
Propoxur	LC-QQQ	20	10
Pyrethrin 1	GC-QTOF	40	20
Pyriproxyfen	LC-QQQ	20	10
Tau-Fluvalinate	GC-QTOF	20	10
Tetrachlorvinphos	LC-QQQ	20	10
Tetramethrin	LC-QQQ	20	10

Table 4 - Estimated wastewater sample allocation with up to nine discrete sampling events for influent and effluent. Up to 30 WWTPs will participate in the project. Up to 10 WWTPs will participate in each sampling event, with each WWTP participating in either the "A", "B", or "C" events. For example, a particular plant might participate in Events 1A, 2A, and 3A, while another plant might participate in Events 1C, 2C, and 3C. Sampling events will be spaced throughout the year to account for seasonal variation (e.g., during dry months and wet months).

Sample Type	Event 1A	Event 1B	Event 1C	Event 2A	Event 2B	Event 2C	Event 3A	Event 3B	Event 3C	Total Samples
Influent	Up to 10	Up to 10	Up to 10	Up to 10	Up to 10	Up to 10	Up to 10	Up to 10	Up to 10	**Up to 90**
Effluent	Up to 10	Up to 10	Up to 10	Up to 10	Up to 10	Up to 10	Up to 10	Up to 10	Up to 10	**Up to 90**

Table 5 - Non-parametric procedures frequently used for comparing paired data, two samples and three or more samples.

Data	Non-Parametric Procedure
Paired data	*Wilcoxon signed-rank test* for uncensored data *Sign test* (modified for ties) for censored data with one RL *Score tests* for censored data with multiple RLs (the PPW test and the Akritas test)
Two samples	*Wilcoxon rank-sum (or Mann-Whitney) test* or *Kolmogorov-Smirnov test* for censored data with one RL *Score tests* for censored data with multiple RLs (the *Gehan test* and generalized *Wilcoxon test*)
Three or more samples in one-way layout	*Kruskal-Wallis test* (for unordered alternative) or *Jonckheere-Terpstra test* (for ordered alternative) for censored data with one RL *Generalized Wilcoxon score test* for censored data with multiple RLs *Multiple comparison* to detect which group is different
Three or more samples in two-way layout	*Friedman's test* (for unordered alternative) or *Page's test* (for ordered alternative) for censored data with one RL Multiple comparison to detect which group is different

8.0 REFERENCES

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