Department of Pesticide Regulation
Environmental Monitoring Branch
Surface Water Protection Program

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STUDY 322: Monitoring Pesticides in Wastewater Influent and Effluent FY22–23

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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, 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 used in pet products (fipronil, permethrin, and imidacloprid) have been detected in sub-sewershed laterals serving dog grooming businesses (Teerlink et al., 2018). Pesticides that are applied outdoors may be transferred to a person's clothing or shoes, and may ultimately be transported down-the-drain through cleaning activities.

Pyrethroids have been detected in treated wastewater effluent of California WWTPs at concentrations that exceed acute toxicity thresholds 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, 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 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 urban and agricultural runoff, it is feasible that variances in regional pest pressures could result in differences in composition and magnitude of pesticide use, and subsequent transport to 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, pesticides such as pyrethroids, fipronil, and imidacloprid are present in effluent at concentrations that exceed toxicity thresholds; however, monitoring studies with consistent and robust analytical methods have been initiated only recently. 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 removal during various wastewater treatment processes.

The monitoring effort described herein builds on DPR'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. 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 presence and concentrations of selected pesticides in wastewater influent and effluent;
2) Evaluate regional and seasonal differences in wastewater pesticide loading to WWTPs;
3) Evaluate magnitude of measured effluent concentrations relative to water quality or aquatic toxicity thresholds;
4) Evaluate influence of sewershed characteristics (i.e., population, contributing land use) on relative pesticide loading;

3.0 PERSONNEL

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

Project Leader: John Wheeler
Reviewing Scientist: Dr. Robert Budd, Ph.D.
Statistician: Dr. Xuyang Zhang, Ph.D.
Laboratory Partner: Department of Toxic Substances Control (DTSC), Environmental Chemistry Laboratory - Pasadena (Contract #20-C0060)
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 the need to collect the necessary data 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, rural), and point of discharge (freshwater or marine). Participating plant information is summarized in Table 1. Volunteer WWTPs will likely 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.

In the initial phase of this monitoring project, SWPP staff conducted retail store surveys to identify pesticide products and associated active ingredients available directly to the consumer with potential for down-the-drain transport including pet products (Vander Werf et al., 2015; Budd & Petters, 2018). SWPP staff used the results from the surveys to create a preliminary list of target analytes. 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. Analytical methods were developed during a previous collaborative project (Contract #18-C0159) with UC Davis. Subsequent research indicated that several pesticides not present in the preliminary list of target analytes (e.g., diuron) had been detected in wastewater effluent in the United States (Sutton et al., 2019). The list of target analytes for the current project is presented in Table 2.

4.3 Sample Collection.

All influent and effluent samples will be collected and shipped by the participating WWTPs. CDPR will provide sampling bottles, shipping coolers, and prepaid shipping labels. 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. If composite sampling is not feasible, "grab" samples may be accepted. Samples will be collected in 125 ml and 250 ml amber glass bottles. Specifically, 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. Influent and effluent sampling will be conducted up to three times at each of the participating WWTPs (Table 3) per year. CDPR may coordinate the timing of sample collection to ensure all samples within the same sampling event are collected within a similar time frame. 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. Influent and effluent samples will be shipped on ice within 24 hours of collection using CDPR-provided coolers and shipping labels to DTSC's Environmental Chemistry Laboratory in Pasadena for analysis. Additionally, effluent samples will be analyzed 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. A chain-of-custody record will be completed and accompany each sample.

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 Contract 20-C0060.

TOC and DOC in samples will be analyzed by SWPP staff at CDPR's Bradshaw Regional Office using a TOC-V CSH/CNS analyzer (Shimadzu Corporation, Kyoto, Japan) (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.

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 DI water will be filtered with each batch of samples.

6.0 DATA ANALYSIS AND REPORTING

Each participating plant will receive the pesticide concentration data from their plant as quickly as they are made available. CDPR will not associate final results with specific plant locations and identities without express written consent of the participating plant. Otherwise, all pesticide concentration data and results will be presented in an anonymized format.

SWPP staff will use various nonparametric and parametric statistical methods to analyze all of the data generated during this study, including site information, general water quality measurements, and pesticide analytical data. The data collected from this project may be used to develop or calibrate a down-the-drain pesticide model.

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. Helsel (2012) illustrated the application of non-parametric procedures to 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) package for R (http://cran.r-project.org/web/packages/NADA/NADA.pdf), and Minitab (http://www.minitab.com/en-us/).

Based on the study objectives, preliminary analysis, and data availability, SWPP staff propose the following statistical procedures for data analysis (Table 4).

1) 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.
2) 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 reporting limits will be censored at the highest limit before proceeding if the test procedure allows only one RL.

Collected data will be summarized in a data report and potentially peer-review journal articles. 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: August 2022 – July 2023
Chemical Analysis: August 2022 – September 2023
Summary Report: January 2024

Table 1 - Summary of participating WWTPs in monitoring study. Additional WWTPs may be added throughout the study to support study objectives.

Facility Treatment	Discharge Point	Number of Plants	Plant Capacity (MGD)*
Secondary	Ocean	12	7.6–450
	Fresh Water	2	6.7–60
Tertiary	Ocean	2	8.5–20
	Fresh Water	7	15–100
	Recycled	2	2
Total		**25**	**2-450**

Table 1 Notes:
**Millions of gallons per day (MGD)*

Table 2 - Pesticides to be monitored for in wastewater influent and effluent, with their respective reporting limit (RL).

Pesticide	Source Type*	Influent RL (ng/L)	Effluent RL (ng/L)
Bifenthrin	LC-QQQ	10	5
Bioallethrin	LC-QQQ	5	2.5
Chlorothalonil	GC-QTOF	5	2.5
Chlorpyrifos	LC-QQQ	1	0.5
Cyfluthrin	GC-QTOF	50	25
Cyhalothrin	GC-QTOF	10	5
Cypermethrin	GC-QTOF	50	25
Cyphenothrin	GC-QTOF	250	125
Deltamethrin	LC-QQQ	25	12.5
Esfenvalerate	GC-QTOF	10	5
Etofenprox	LC-QQQ	1	0.5
Fenpropathrin	LC-QQQ	5	2.5
Fipronil	GC-QTOF	5	2.5
Fipronil amide	GC-QTOF	5	2.5
Fipronil desulfinyl	GC-QTOF	5	2.5
Fipronil desulfinyl amide	GC-QTOF	5	2.5
Fipronil sulfide	GC-QTOF	5	2.5
Fipronil sulfone	GC-QTOF	10	5
Imidacloprid	LC-QQQ	5	2.5
Novaluron	GC-QTOF	5	2.5
Permethrin	LC-QQQ	50	25
Phenothrin	GC-QTOF	5,000	2,500
Prallethrin	LC-QQQ	5	2.5
Propoxur	LC-QQQ	1	0.5
Pyrethrin 1	LC-QQQ	5	2.5
Pyriproxyfen	LC-QQQ	1	0.5
Tau-Fluvalinate	LC-QQQ	5	2.5
Tetrachlorvinphos	LC-QQQ	10	5
Tetramethrin	LC-QQQ	10	5

**Source Type:*
GC-QTOF = Gas chromatography with quadrupole time-of-flight mass spectrometry
LC-QQQ = Liquid chromatography with triple quadrupole mass spectrometry

Table 3 - Estimated wastewater sample allocation with up to nine discrete sampling events for influent and effluent.

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 85**
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 85**

Table 3 Notes:
** 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).*

Table 4 - 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

References

Budd, R., & Petters, K. (2018). Survey of pesticide products sold in retail stores in Northern California, 2017. *California Department of Pesticide Regulation Analysis memo. Available at: https://www.cdpr.ca.gov/docs/emon/pubs/ehapreps/analysis_memos/pesticide_product_survey_120718.pdf*.

Ensminger, M. (2013a). *Standard Operating Procedure METH011.00 Water TOC Analysis Using the Shimadzu TOC-VCSN and ASI-V Autosampler*. Sacramento, CA

Ensminger, M. (2013b). *Standard Operating Procedure METH010.01 Analysis of Whole Sample Suspended Sediments in Water*. Sacramento, CA

Ensminger, M., Budd, R., Kelley, K., & Goh, K. (2013). Pesticide occurrence and aquatic benchmark exceedances in urban surface waters and sediments in three urban areas of California, USA, 2008–2011. *Environmental Monitoring and Assessment*, 1-14. https://doi.org/10.1007/s10661-012-2821-8

Helsel, D. R. (2012). *Statistics for censored environmental data using Minitab and R* (2nd ed.). John Wiley and Sons.

Sadaria, A., Sutton, R., Moran, K., Teerlink, J., Brown, J., and R. Halden (2017). Passage of fiproles and imidacloprid from urban pest control used through wastewater treatment plants in northern California, USA. *Environmental Toxicology & Chemistry*, *36*(6), 1473-1482.

Sutton, R., Xie, Y., Moran, K. D., & Teerlink, J. (2019). Occurrence and sources of pesticides to urban wastewater and the environment. In *Pesticides in Surface Water: Monitoring, Modeling, Risk Assessment, and Management* (pp. 63-88). ACS Publications.

Teerlink, J., Hernandez, J., & Budd, R. (2017). Fipronil washoff to municipal wastewater from dogs treated with spot-on products. *Science of the Total Environment*, *599–600*, 960-966. https://doi.org/https://doi.org/10.1016/j.scitotenv.2017.04.219

Teerlink;, J., Xie;, Y., Budd;, R., & Young, T. (2018). Loading of Pesticides to Wastewater Catchments from Flea and Tick Topical Products. SETAC North America 39th Annual Meeting, Sacramento CA.

Vander Werf, R., Aldana, A., Teerlink, J., & Budd, R. (2015). Retail Store Survey of Consumer-Use Indoor Pesticide Products, 2014. *California Department of Pesticide Regulation Analysis memo. Available at: http://www.cdpr.ca.gov/docs/emon/pubs/ehapreps/analysis_memos/retail_memo_consumer_survey.pdf*.

Weston, D. P., Ramil, H. L., & Lydy, M. J. (2013). Pyrethroid Insecticides in Municipal Wastewater [Article]. *Environmental Toxicology and Chemistry*, *32*(11), 2460-2468. https://doi.org/10.1002/etc.2338

Xie, Y., Budd, R., Teerlink, J., Luo, Y., & Singhasemanon, N. (2021). Assessing pesticide uses with potentials for down-the-drain transport to wastewater in California. *Sci Total Environ*, *773*, 145636. https://doi.org/10.1016/j.scitotenv.2021.145636