Department of Pesticide Regulation
Environmental Monitoring Branch
Surface Water Protection Program

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STUDY 322: Monitoring Pesticides in Wastewater Treatment Plant Influent and Effluent (2026)

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October 8, 2025

1.0 INTRODUCTION

The occurrence of pesticides in treated wastewater treatment plant (WWTP) effluent at concentrations that exceed aquatic toxicity thresholds has been documented 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, topical flea and tick treatment products applied to dogs have been shown to wash off during dog bathing (Teerlink et al., 2017), as well as during owner handwashing and dog bed laundering (Perkins et al., 2024). 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). Also, it is hypothesized that pesticides 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 WWTP effluent of California WWTPs at concentrations that exhibited sub-lethal effects for sensitive invertebrates (Weston et al., 2013). A 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). Another study found that some pesticides were partially removed by advanced treatment processes, but removal percentages were highly variable (Lau and Mitch, 2024). 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 WWTP 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 plausible that regional variation in pest pressures could result in differences in pesticide use, resulting in subsequent regional differences in composition of pesticides entering the waste stream (Ensminger et al., 2013). WWTPs have a wide range of treatment capabilities before discharging effluent. The vast majority of WWTPs are equipped with at least secondary treatment, and many have additional tertiary processes. Treated wastewater may be additionally disinfected with ultraviolet (UV) radiation or chlorine prior to leaving the WWTP. 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 (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 waste stream. Information gained from this effort will allow assessment of differences in concentrations based on region, surrounding land use, and treatment level (i.e., secondary, tertiary). 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 (changes made between this protocol and the prior year's protocol are presented in Table 1). Subsequent year protocols may incorporate additional study objectives.

2.0 OBJECTIVES

The overall goal of this project is to assess pesticide concentrations found in WWTP influent and effluent in California. Specific objectives include: Evaluate regional and seasonal variability in wastewater pesticide loading to WWTPs; Evaluate the influence of grouping variables such as sewershed and facility characteristics (e.g., population, contributing land use, facility treatment level) on relative pesticide loading; 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 DPR'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: WWTPs throughout California
Please direct questions regarding this study to John Wheeler, Senior Environmental Scientist (Specialist), by email at John.Wheeler(at)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 WWTP management and technical staff. Participating WWTPs will span a wide range of comparative parameters, including geographic region, capacity (measured in gallons treated per day), treatment capability (i.e., secondary or tertiary), final disinfection method (i.e., disinfectant process or chemical), surrounding land use patterns (e.g., urban, agricultural), and point of discharge (i.e., freshwater or marine). Information regarding current WWTP participants is summarized in Table 2. Volunteer WWTPs may be asked to commit to participating in approximately three sampling events per year, for a period of 1 to 3 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 at any given time. Additional WWTPs may be included as participation in the project increases.

4.2 Pesticides for Analysis.

Target analytes were chosen 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 WWTP 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. In 2023, SWPP staff performed a review of recently registered pesticide products with down-the-drain transport potential. This search yielded five AIs of interest which were not currently on the list of analytes (dinotefuran, S-methoprene, S-indoxacarb, fluralaner, and flumethrin). Surface Water staff then requested that DTSC conduct method development to analyze for these compounds. DTSC successfully added these five compounds to the analyte list, and has begun analyzing for these compounds (beginning with samples collected in September 2023).

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. The list of target analytes for the current monitoring project is presented in Table 3.

4.3 Sample Collection.

All influent and effluent samples will be collected and shipped by the participating WWTPs. Sampling bottles, shipping coolers, ice packs, and prepaid FedEx overnight shipping labels will be provided by DPR. Sample collection methods will follow methods consistent with individual

WWTP collection protocols. 24-hour composite samples (either flow-weighted or time-weighted) are preferred for both influent and effluent, when feasible. If composite sampling is not feasible, “grab” samples will be accepted. Influent samples will be collected before primary treatment (WWTPs are usually required to sample their influent for other purposes; the influent sampling point used for those other purposes is typically also acceptable for this project). 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 WWTPs will be asked to complete a chain-of-custody (COC) form provided by DPR, which will include space to record details such as sampling date/time and collection method.

Samples will be collected in pre-labeled bottles provided by DPR (typically: 125 ml, 500 ml, and/or 1L amber glass). Most WWTPs will be asked to collect approximately 3 to 4L of effluent per sampling event. The exact sample volumes requested may change over time, but will always be discussed and confirmed with WWTPs several weeks ahead of the sampling event. All sampling events will include effluent, while approximately half of sampling events will also include influent (Table 4). When influent samples are requested, the specific request will typically be up to 375 ml (up to 3 x 125 ml bottles) from most WWTPs. The different volumes of influent and effluent are necessary due to differences in the analytical methods used for these two sample matrices. Some WWTPs may be asked to collect greater volumes of influent and/or effluent than those described above, for laboratory quality control purposes (details will be discussed ahead of time). Additionally, under special circumstances or sampling events, DPR may request additional samples and/or samples collected in different sized bottles; details will be discussed with WWTPs ahead of the planned sampling event.

Samples will be shipped on wet ice or ice packs (provided by DPR) within 24 hours of collection, using DPR-provided coolers and prepaid FedEx overnight shipping labels, to DTSC’s Environmental Chemistry Laboratory in Pasadena for pesticide analysis. Additionally, effluent samples may 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 samples (e.g., daily flow data) may be provided by individual WWTPs.

Sampling will be conducted up to three times per year at each of the participating WWTPs (Table 4). DPR 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 WWTPs 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 during the standard work week to DTSC’s Environmental Chemistry Laboratory. Sampling events will be spaced throughout the year to account for possible seasonal variation in pesticide concentrations. DPR 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 2025 monitoring protocol (dated April 28, 2025), as shown in Table 1.

Table 1 - Changes made from the 2025 protocol.

Section of Document	Description of Change(s) Made
1.0 Introduction	Added citations to newly published research, when available.
2.0 Objectives	Added text to clarify that facility treatment level may be used as a grouping variable to evaluate any differences in pesticide concentrations between secondary and tertiary facilities.
4.3 Sample Collection	Added further details regarding sampling events (e.g., the specific sample volumes that WWTPs will usually be asked to provide).
5.0 Chemical Analysis	Updated this section to reflect the most recent analysis protocols utilized by SWPP staff.
7.0 Timeline	Updated the timeline to reflect the current project year.
Table 3	Updated the table to reflect the current effluent reporting limits, which are lower than those reported in the 2025 protocol for all analytes.
Table 4	Updated the table to reflect the 2026 sampling plan.

5.0 CHEMICAL ANALYSIS

Samples will be analyzed for pesticides by DTSC's Environmental Chemistry Laboratory in Pasadena according to the methods developed under DPR 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 in three separate documents on DPR's Reports Directory (https://www.cdpr.ca.gov/reports-directory/?cat=114&yr=2022&rt=guide). The three documents are titled as follows: "Automated Solid-Phase Extraction (SPE) for Pesticides," "Pesticide Analysis by Gas Chromatography / Quadrupole Time-of-Flight Mass Spectrometry (GC/QToF)," and "Pesticide Analysis by Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-MS-MS)."

The TOC and DOC in effluent samples will be analyzed by SWPP staff at DPR's Bradshaw Regional Office using an Elementar TOC Vario Cube (Elementar Analysensysteme GmbH, Langenselbold, Germany) based on the protocol by Zoerner (2025). Before analysis of every sample set, lab blanks and calibration standards will be run to ensure the quality of the TOC and DOC data.

Staff from SWPP will analyze TSS in effluent samples by filtering the 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 suspended solids retained on the filter (Ensminger, 2013). 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.

Pesticide monitoring data are typically 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). Staff from SWPP 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*) and *EnvStats* packages for R. In addition, SWPP staff will use non-parametric methods for the analysis of concentration differences among different factors and temporal trends.

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.

DPR staff will provide each participating WWTP with a copy of their 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, analysis memos, peer-reviewed journal articles, presentations), DPR will *not* identify participating WWTPs / agencies (including identifying characteristics such as discharge coordinates) or associate pesticide concentrations with specific WWTP locations or identities without express written consent of the participating plant. Otherwise, all participating WWTPs / agencies will remain anonymous. Prior to publication, participating WWTPs / agencies will be granted the opportunity (minimum of 30 calendar days) to review public-facing materials. However, to expedite the process, DPR may request permission in advance for the use of data in certain types of reports. In addition, for WWTPs that are newly added to the monitoring project, DPR may ask WWTP staff to complete a short questionnaire to assess which type(s) of data use are permissible for pesticide data obtained from the samples collected from the WWTP.

7.0 TIMELINE

The dates shown below are estimated and are subject to change.
WWTP Sampling: January 2026 – December 2026.
Chemical Analysis: January 2026 – April 2027.
Summary Report: October 2027.

*Table 2 - Summary of WWTPs currently participating in the project. For some WWTPs, a portion of effluent is reused/recycled (e.g., groundwater recharge, irrigation) while the remainder is discharged to a water body. In this table, WWTPs are classified based on the majority of the effluent volume (i.e., if >50% of the effluent is reused/recycled, the WWTP would be classified as "Reused/Recycled" in this table). Note: the values presented in this table are **not** considered to be "criteria" for study participation (i.e., WWTPs that fall outside the capacity ranges shown in the table may still be considered for participation in the project).*

Treatment Level	Discharge Point	Number of WWTPs	Plant Capacity (millions of gallons per day; MGD)
Secondary	Ocean/Bay	13	6.7 to 400
Secondary	Reused/Recycled	1	0.2
Tertiary	Ocean/Bay	1	39
Tertiary	Fresh Water	9	7.5 to 180
Tertiary	Reused/Recycled	4	6.5 to 18
Total	**All of the above**	**28**	**0.2 to 400**

Table 3 - Pesticides to be monitored in WWTP 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.

Pesticide	Instrumentation	Influent RL (µg/L)	Effluent RL (µg/L)
alpha-Cypermethrin	GC-QTOF	0.003	0.001
beta-Cyfluthrin	GC-QTOF	0.008	0.001
Bifenthrin	GC-QTOF	0.031	0.004
Chlorothalonil	GC-QTOF	0.016	0.002
Chlorpyrifos	GC-QTOF	0.016	0.002
Cyfluthrin	GC-QTOF	0.016	0.002
Cypermethrin	GC-QTOF	0.016	0.002
Cyphenothrin	GC-QTOF	0.078	0.010
Deltamethrin	GC-QTOF	0.078	0.010
Dinotefuran	LC-QQQ	0.016	0.002
Esfenvalerate	LC-QQQ	0.031	0.010
Etofenprox	LC-QQQ	0.016	0.005
Fenpropathrin	LC-QQQ	0.031	0.010
Fipronil	GC-QTOF	0.016	0.002
Fipronil amide	GC-QTOF	0.016	0.002
Fipronil desulfinyl	GC-QTOF	0.016	0.002
Fipronil desulfinyl amide	GC-QTOF	0.016	0.002
Fipronil sulfide	GC-QTOF	0.016	0.002
Fipronil sulfone	GC-QTOF	0.016	0.002
Flumethrin	LC-QQQ	0.031	0.010
Fluralaner	LC-QQQ	0.016	0.002
gamma-Cyhalothrin	GC-QTOF	0.010	0.001
Imidacloprid	LC-QQQ	0.016	0.002
lambda-Cyhalothrin	GC-QTOF	0.031	0.004
Novaluron	LC-QQQ	0.016	0.002
Permethrin	LC-QQQ	0.391	0.100
Phenothrin	GC-QTOF	0.783	0.100
Prallethrin	GC-QTOF	0.016	0.002
Propoxur	LC-QQQ	0.016	0.002
Pyrethrin 1	GC-QTOF	0.031	0.004
Pyriproxyfen	LC-QQQ	0.016	0.002
S-Bioallethrin	GC-QTOF	0.016	0.002
S-Indoxacarb	LC-QQQ	0.016	0.002
S-Methoprene	LC-QQQ	0.063	0.008
Tau-Fluvalinate	GC-QTOF	0.016	0.002
Tetrachlorvinphos	LC-QQQ	0.016	0.002
Tetramethrin	LC-QQQ	0.016	0.002

Table 4 - Estimated wastewater sample allocation with up to six discrete sampling events in the calendar year. Each sampling event will include effluent sampling, while half of the events will also include influent sampling. Up to 30 WWTPs will participate in the project at any given time. Up to 15 WWTPs will participate in each sampling event, with each WWTP participating in either the "A" or "B" events. For example, a particular WWTP might participate in Events 11A, 12A, and 13A, while another WWTP might participate in Events 11B, 12B, and 13B. Sampling events will be spaced throughout the year to account for seasonal variation (e.g., during dry months and wet months). Numbers and dates shown in the table are approximate, and may change based on several factors (e.g., laboratory capacity, scheduling constraints).

Sample Type	Event 11A (Jan 2026)	Event 11B (March 2026)	Event 12A (April 2026)	Event 12B (July 2026)	Event 13A (Sept 2026)	Event 13B (Nov 2026)	Total Samples
Influent	None	15	15	None	None	15	**45**
Effluent	15	15	15	15	15	15	**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 and/or Peto-Peto 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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