Emerging contaminants in wastewater and receiving surface water environments

Technical Report

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Ecological risk and emerging contaminants, EPA Science

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Executive summary

The purpose of this project was to identify the prevalence and frequency of emerging contaminants in wastewater streams, and their receiving surface water environments in Victoria. Additionally, this project aimed to develop a preliminary understanding of the use of bioanalytical tools (i.e. bioassays) and effects-based monitoring (EBM) in assessing the risks of emerging contaminants to the environment, as an additional line of evidence complementary to targeted chemical analyses. Overall, this project contributes towards improving the state of knowledge on emerging contaminants and enabling the Environment Protection Authority Victoria (EPA) to identify new, and more holistic tools to assess and manage risks of emerging contaminants to ecosystem health and function.

EPA collected and analysed influent (raw sewage) and effluent water (treated wastewater) samples at 4 wastewater treatment plants (WWTPs) across Victoria, and adjacent effluent-receiving surface water streams. Specifically, water samples were collected up and downstream from the discharge point, and at the discharge point. Additionally, two surface water streams with no specific wastewater point inputs were sampled as reference sites (total of 24 surface water samples). Samples were analysed for a range of emerging contaminants, including artificial sweeteners, endocrine disrupting chemicals (EDCs), per- and polyfluoroalkyl substances (PFAS), pharmaceuticals and personal care products (PPCPs), pesticides, and phthalates. The battery of bioassays chosen for the study were indicative of bacterial toxicity, photosynthesis inhibition, estrogenic activity, oxidative stress response, activation of the aryl hydrocarbon (AhR) receptor and genotoxicity. The bioassays were applied to all samples (influent, effluent and surface water), as a measure of overall contaminant toxicity and toxicity from specific contaminant groups.

From 32 samples collected, a total of 643 emerging contaminants were analysed in each sample, of which 143 contaminants were detected above the limit of reporting. Across all samples, 4 artificial sweeteners, 11 EDCs, 36 pesticides, 18 PFAS, 6 phthalates, and 68 PPCPs were detected. For all contaminant groups other than PFAS, on average, concentrations were lower in effluent water than in raw sewage influent, which was consistent with results from a previous EPA publication (EPA Publication 2054; EPA, 2023).

For PPCPs, concentrations ranged from <0.005 to 200 µg/L in influent, from <0.005 to 12 µg/L in effluent, from <0.005 to 0.17 µg/L in surface water upstream of wastewater discharge, and from <0.005 to 0.99 µg/L in surface water downstream of wastewater discharge. There are currently no guideline values for PPCPs in Australia (ANZG, 2024).

For artificial sweeteners, concentrations ranged from <0.005 to 32 µg/L in influent, from <0.005 to 34 µg/L in effluent, from <0.005 to 0.37 µg/L in surface water upstream of wastewater discharge, and from <0.005 to 8.8 µg/L in surface water downstream of wastewater discharge. There are currently no guideline values for artificial sweeteners in Australia (ANZG, 2024).

For EDCs, concentrations ranged from <0.002 to 23 µg/L in influent, from <0.002 to 24 µg/L in effluent, were not detected (<0.002 µg/L) in surface water upstream of wastewater discharge, and from <0.002 to 4.8 µg/L in surface water downstream of wastewater discharge. Of the EDCs detected, guideline values exist only for Bisphenol A, with none of the detected concentrations exceeding the most conservative 99% species protection exposure concentration of 0.78 µg/L for freshwater (ANZG, 2024).

For pesticides, concentrations ranged from <0.01 to 3.2 µg/L in influent, from <0.01 to 0.36 µg/L in effluent, from <0.01 to 0.11 µg/L in surface water upstream of wastewater discharge, and from <0.01 to 0.1 µg/L in surface water downstream of wastewater discharge. Guideline values were exceeded for metolachlor and metsulfuron-methyl for freshwater (ANZG, 2024).

For PFAS, concentrations ranged from <0.0002 to 0.41 µg/L in influent, from <0.0002 to 0.44 µg/L in effluent, from <0.0002 to 0.048 µg/L in surface water upstream of wastewater discharge, and from <0.0002 to 0.097 µg/L in surface water downstream of wastewater discharge. There were exceedances of freshwater guideline values for PFOS (ANZG, 2023), but not for PFOA or the sum of PFOA and PFHxS (HEPA, 2022).

For phthalates, concentrations ranged from <0.01 to 64 µg/L in influent, from <0.01 to 7.6 µg/L in effluent, from <0.01 to 0.23 µg/L in surface water upstream of wastewater discharge, and from <0.01 to 0.24 µg/L in surface water downstream of wastewater discharge. The only exceedances of freshwater guideline values was for concentrations of DEHP detected in effluent, but no guideline values were exceeded for phthalates detected in surface water samples (ANZG, 2024; NHMRC and NRMMC, 2011).

For the bioassays, all wastewater influent and effluent samples showed a response in assays indicative of bacterial toxicity, photosynthesis inhibition, estrogenic activity, oxidative stress response and activation of AhR. However, most of these bioassay responses observed in wastewater samples were consistent with the range of effects typically seen in wastewater across other studies, and may not necessarily indicate an increased risk to the receiving environment. Further investigation into bioassay responses at the point of exposure (i.e., in surface water) would be required to fully assess ecological risks to the environment from effluent discharges. In contrast, none of the samples were genotoxic. Three of the four WWTPs sampled (Sites B–D) were able to remove over 80% of bacterial toxicity, photosynthesis inhibition, estrogenic activity and oxidative stress response. Lower removal efficiency was consistently observed for one of the WWTPs (Site A), with particularly poor removal of estrogenic activity and AhR activity.

Surface water samples showed a range of responses in bioassays. For example, bacterial toxicity assays showed a response over the limit of detection in only a few samples (3 out of 20), while most samples for the photosynthesis inhibition assay showed effects close to the limit of detection. Estrogenic activity, AhR activity and induction of the oxidative stress response were more commonly observed in surface water, with the effect in some of the samples exceeding the ecological effects-based threshold (ecoEBT), which are ecological guideline values derived specifically for bioassays as general indicators for water quality (but are not enforced by regulations). In contrast, the control reference site samples did not induce a response in any of the bioassays.

Overall, the bioassays show that the water quality upstream and downstream of the WWTPs was acceptable for the majority of studied endpoints (other than estrogenic activity), with the observed effects similar to previously reported activity for surface water globally.

This study shows that effects-based methods in combination with targeted chemical analyses provides a comprehensive toolkit to assess the risks of emerging contaminants to Victoria's waterways. However, this preliminary dataset covers only a small fraction of WWTPs and the types of treatment processes at these sites. Further work is warranted to improve our understanding of the treatment efficacy at WWTPs for emerging contaminants and how bioassay responses may differ across various types of wastewater treatment processes.

Contents

Background

In 2021, EPA conducted a study on the presence of emerging contaminants in recycled water, and the reduction or removal of these compounds over various different treatment processes (i.e., treatment trains) at wastewater treatment plants (WWTPs) (see EPA publication 2054; EPA, 2023). In that study, influent and effluent samples were analysed at 31 WWTPs across Victoria for a range of emerging contaminant groups, including pharmaceuticals and personal care products (PPCPs), endocrine disrupting chemicals (EDCs), industrial compounds, pesticides, disinfection byproducts, phenols, and per- and polyfluoroalkyl substances (PFAS). Overall, 180 emerging contaminants were detected in influent and effluent samples. For most of these compounds, concentrations were lower in effluent than in influent, although reduction during treatment was found to be compound specific. The 2021 study identified some contaminants (e.g. pharmaceuticals) that pass-through treatment trains relatively untreated. Because treated wastewater can be discharged into waterways, the untreated contaminants entering surface water environments can pose a risk to wildlife and ecosystem health.

Emerging contaminants in the environment present a regulatory challenge, as their prevalence, concentrations, and potential risks are not generally well understood (Geissen et al., 2015; Noguera-Oviedo and Aga, 2016). Many emerging contaminants do not have water quality guidelines because there is insufficient knowledge of their toxicological properties along with their combined mixture effects. Water quality and the risks of emerging contaminants to the environment is typically assessed using targeted chemical analysis for a subset of key groups and are often limited to several hundred substances. However, effluent discharges are known to have a mixture of thousands of chemicals present, which are yet to be targeted by traditional analytical methods or are below current detection limits (Neale et al., 2020). In addition, current environmental risk assessments and standards do not typically account for the toxicity of mixture effects. Therefore, a more holistic approach is required for water quality monitoring and assessment of risks of emerging contaminants.

One such tool is effects-based monitoring (EBM), via the use of bioassays. Globally, EBM has been identified as one of the most promising tools to improve assessment of risks from emerging contaminants in wastewater effluents (Enault et al., 2023; Neale et al., 2020; van der Oost et al., 2017). A combination of both targeted chemical analyses and bioassays provides a holistic way to assess the impacts of wastewater effluent discharge to the receiving environment (e.g. Leusch et al., 2018). Like individual chemical guideline values, the observed effect in a bioassay can be compared to bioassay specific effects-based trigger values (EBTs), to determine whether chemical water quality is acceptable or has potential risks of harm. One important advantage of bioassays over targeted chemical analyses in the assessment of environmental risks is the ability to discern specific biological effects of contaminant mixtures, helping to identify direct impacts to exposed organisms. Despite this, to date, EBM have primarily been applied in a research context, with less uptake by the water industry and regulators, partly due to concerns regarding reliability and interpretation of results (Neale et al., 2023a).

The overall aim of this study was to trial EBM techniques to assess the potential risk of emerging contaminants in effluent discharged into surface waters. Accordingly, the objectives of this study were to:

1) Determine the presence and concentrations of emerging contaminants in wastewater influent and effluent from 4 WWTPs, and adjacent up- and downstream surface water sites.
2) Estimate percent reduction of emerging contaminants across sampled sites and respective treatment technologies.
3) Trial the use of an EBM assessment as a weight-of-evidence process to determine risks to the receiving environment from emerging contaminants in wastewater discharges.

Methods

3.1. Sampling locations and design

EPA sampled sewage influent and effluent from 4 WWTPs across Victoria (Sites A–D), along with surface water from adjacent effluent-receiving waterways, between 17th April 2024 and 12th June 2024. A fifth site (Site E) was sampled for only surface water from a WWTP effluent-receiving waterway due to logistical constraints, as were two reference waterways (Sites F & G) with no known associated WWTP discharge. See Table 1 below for a list of treatment trains and treatment processes associated with each site.

At each WWTP site, surface water was sampled from 4 locations: at the WWTP discharge point, then at locations downstream and upstream of the discharge point (~500 m–2 km away), and finally a second further downstream location within the license-designated mixing zone (>2–15 km away from discharge) (Figure 1). For each reference site, surface water samples were collected from an upstream and downstream location. Wastewater influent and effluent were collected on-site at the WWTP. Therefore, there were 6 sampling points per WWTP site for Sites A–D, with 4 sampling points for Site E and 2 sampling points for each of the reference waterways, Sites F & G.

Table 1. Respective treatment trains and processes at each sampled site.

Site sampled	Treatment train	Treatment processes
Site A	TT4	Activated Sludge Process (ASP) with extended aeration, ultraviolet (UV) disinfection
Site B	TT7	ASP, UV disinfection
Site C	TT7	ASP, secondary sedimentation, UV disinfection
Site D	TT14	ASP with extended aeration, secondary sedimentation, UV disinfection
Site E	TT1	Lagoon
Site F	-	Reference site
Site G	-	Reference site

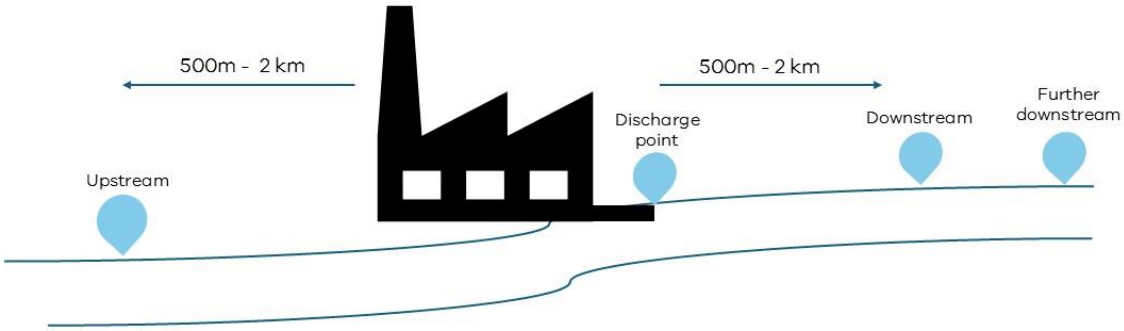

Figure 1. Schematic of the sampling design at each effluent-receiving site for this study.

3.2. Sample collection

All samples were collected and stored in accordance with EPA publication IWRG 701 (EPA, 2009) and PFAS NEMP 2.0 (HEPA, 2020). For raw influent and effluent, samples were collected as per methods outlined in EPA Publication 2054 (EPA, 2023). Briefly, a 24-hour composite of raw sewage influent and treated effluent streams at each WWTP site (Sites A–D) was collected via autosampler (set at 15-minute collection intervals).

Surface water samples were collected from effluent-receiving waterways (Sites A–E) and reference waterways (Sites F & G) using the grab water sampling methods previously described (EPA, 2023). Samples were collected with stainless-steel sampling poles, in both amber glass bottles and HDPE bottles pre-rinsed with solvent or ultrapure water. Samples were transported on ice to Leeder Analytical (Melbourne, Victoria) for chemical analyses within 48 h of collection and analysed within 3-5 days of collection.

3.3. Emerging contaminants chemical analysis

Samples were analysed for 11 artificial sweeteners, 15 EDCs, 445 pesticides, 46 PFAS, 19 phthalates and 109 PPCPs.

3.4. Bioassay analysis

For bioassay analyses, samples were transported on ice to the Australian Rivers Institute, Griffith University (Southport, Queensland) within 48 h of collection. A battery of six bioassays were applied to screen samples for chemical-associated toxicity. Briefly, these assays were: BLT-Screen for bacterial toxicity due to a range of general contaminants, IPAM assay for photosynthesis inhibition and toxicity to algae due to herbicides and pesticides, ER-GeneBLAzer for estrogenic and anti-estrogenic EDCs, HiTMiN assay for genotoxic and cytotoxic chemicals, ARE-GeneBLAzer for oxidative stress due to general contamination, and AhR-CAFLUX for dioxin-like compounds and pesticides. See below for a brief

description of each bioassay, and Appendix A for detailed methodological information on each bioassay.

3.4.1. BLT-Screen assay (general toxicity to bacteria)

The BLT-Screen assay is a measure of toxicity to bacteria that covers a wide range of organic micropollutants and is generally an indicator for overall contaminant toxicity and water quality.

3.4.2. IPAM assay (photosynthesis inhibition due to herbicides)

The IPAM assay quantifies inhibition of photosynthesis at 2 h (2h-IPAM) and 24 h (24h-IPAM), determines overall toxicity to algae, and is usually well correlated with herbicide activity. Typically, more chemicals in addition to PSII herbicides contribute to photosynthesis inhibition after 24 h, and thus, the 24h-IPAM more broadly measures adverse effects of pesticides.

3.4.3. ER-GeneBLAzer assay (estrogenic activity from EDCs)

The ER-GeneBLAzer assay measures estrogen agonism and antagonism caused by EDCs, which can interfere with the normal function of the endocrine system in animals and humans.

3.4.4. ARE-GeneBLAzer assay (general oxidative stress response)

The ARE-GeneBLAzer assay is a measure of oxidative stress, which can be caused by a variety of chemicals and chemical groups. Similar to the BLT-Screen above, a response in the ARE-GeneBLAzer assay can be benchmarked to previously established water quality data to provide an indicator of overall water quality and risks of harm to the environment.

3.4.5. AhR-CAFLUX assay (presence of dioxin-like chemicals)

The AhR-CAFLUX assay measures the induction of the aryl hydrocarbon receptor (AhR), which is indicative of the presence of dioxin-like chemicals in addition to other chemical groups such as pesticides and polycyclic aromatic hydrocarbons (PAHs), which can be highly toxic and persistent.

3.4.6. HiTMiN assay (genotoxicity)

The HiTMiN assay relies on high-throughput cell imaging to analyse micronucleus formation in cells. Micronucleus formation is a recognised measure of DNA damage (i.e. genotoxicity), which has the potential to lead to malignant transformation.

3.5. Quality assurance and quality control (QAQC)

The QAQC procedures for field sampling included collection and analysis of trip blanks, field duplicates, and field blanks, consistent with EPA publication IWRG701 (EPA, 2009) and PFAS NEMP 2.0 (HEPA, 2020). No contaminants were detected above the limit of reporting (LOR) in field or trip blanks.

Laboratory QAQC parameters used to validate analytical results for chemical analysis encompassed method blanks, laboratory control standards (LCS), duplicates and matrix spikes. LCS recoveries were within 46–130% for samples, and acceptable spike recoveries were within the range of 50–150%. Method blanks showed no background contamination above LOR. All contaminant concentrations reported are internal standard corrected and reported as µg/L. The laboratory analysing the samples (Leeder Analytical) is accredited for analytical testing by the National Association of Testing Authorities (NATA) and is ISO/IEC 17025 compliant. See Appendix A for QAQC protocols for bioassays.

3.6. Data analysis

Summary results and statistical analyses were conducted in R version 4.4.1 (R Core Team, 2023). If analysed concentrations of chemicals were below the LOR, for calculation of summary statistics such as means, zero values were used. Concentration means, maximum, minimum, and standard deviations were calculated for each contaminant group at each sampling location across all sites.

3.7. Percent reduction (%R)

To understand how different chemicals and chemical groups behaved during the wastewater treatment processes, we estimated the change in concentration from influent to effluent samples for individual chemicals and chemical groups across WWTP sites (Sites A–D). Estimates in percent reduction (%R) of emerging contaminants in wastewater were calculated for each site (A–D) undertaking the same methods as reported in EPA Publication 2054 (EPA, 2023) and as per methods presented in Luo et al. (2014).

Percent reduction (%R) was estimated for all individual chemicals detected above the LOR in wastewater influent and effluent., as a percentage of the concentration remaining after moving through wastewater treatment (Equation 1):

$$Percent\ reduction\ (\%R) = 100 \times \left(\frac{C_{inf} - C_{eff}}{C_{inf}}\right) \quad \text{(Equation 1)}$$

where c_{inf} is the concentration of an individual chemical detected in influent, and c_{eff} is the concentration of an individual chemical detected in effluent. A positive %R estimate indicates a decrease in the concentration from influent to effluent (and thus removal via wastewater treatment), and a negative %R estimate indicates an increase in concentration from influent to effluent.

It is important to note that chemicals that were not detected in influent (<LOR), but were detected in effluent, were excluded from calculation due to division by zero errors.

3.8. Guidelines

Where available, we compared concentrations of detected compounds in effluent and surface waters with ecological and/or human-health based guideline values from Australian sources. The primary guidelines relevant to this study are ecological/ecosystem-based guidelines for water quality, such as the Australian and New Zealand governments (ANZG) toxicant default guideline values for aquatic ecosystem protection (ANZG, 2024), and the National Environmental Management Plan on PFAS (PFAS NEMP v3.0; HEPA, 2022). Additionally, there are human health-based guidelines for recreational water such as the guidelines for managing risks in recreational water (NHMRC, 2008), and of lesser relevance to the sampling conducted in this study, for drinking water such as the Australian Drinking Water Guidelines 6 (NHMRC and NRMMC, 2011).

The observed effect in a bioassay can be compared to bioassay specific effects-based trigger values (EBTs), which are similar to guideline values for known chemicals, to determine whether chemical water quality is acceptable or has potential risks of harm (Escher et al., 2018). Here, we use EBTs derived for protection of ecosystem health (i.e., ecological effects-based trigger values; ecoEBT) to determine potential risks to the surface water environment due to WWTP discharge. See Appendix A for detailed information on sources of ecoEBTs.

Results

4.1. Emerging contaminants in wastewater influent and effluent

4.1.1. Pharmaceuticals and personal care products (PPCPs)

For PPCPs, concentrations in influent ranged from <0.005 to 200 µg/L, and from <0.005 to 12 µg/L in effluent (Table 2). Of the 109 PPCPs analysed in wastewater, 67 were detected in influent, and 55 in effluent, including some illicit substances.

In influent, the highest PPCP concentrations detected was for acetaminophen (a.k.a. paracetamol; 200 µg/L), followed by caffeine (100 µg/L), metformin (41 µg/L), gabapentin (21 µg/L), octocrylene (21 µg/L), and ibuprofen (15 µg/L). Of the 67 detected PPCPs in influent, 32 PPCPs were detected in 100% of influent samples (see Appendix B for list of PPCPs detected). Furthermore, PPCPs detected only in influent but not in effluent included: amphetamine, aspirin, dicloxacillin, minocycline, paraben ethyl, paraben methyl, paraben propyl, sertraline, tetracycline, theophylline, triclosan, triclocarban, and warfarin. Across WWTP sites, the mean concentrations of PPCPs in influent from highest to lowest were, as follows: Site B > Site C > Site A > Site D, with the highest maximum concentration in influent observed at Site B (Table 3).

In effluent, the highest concentrations of PPCPs detected were for metformin (12 µg/L), valsartan (6.4 µg/L), lamotrigine (3 µg/L), gabapentin (2.7 µg/L), phenytoin (2.3 µg/L) and flurosemide (1.8 µg/L). Of the 55 PPCPs detected in effluent, 28 were detected in 100% of effluent samples. The only PPCP detected in effluent but not influent was cyclophosphamide. Across WWTP sites, the mean concentrations of PPCPs in effluent from highest to lowest were as follows: Site A > Site B > Site D > Site C, with the highest maximum concentration in effluent observed at Site B (Table 3).

4.1.2. Artificial sweeteners

Concentrations of artificial sweeteners in influent ranged from <0.005 µg/L up to 32 µg/L, and in effluent from <0.005 µg/L up to 34 µg/L (Table 2). Of the 11 artificial sweeteners analysed in wastewater, 4 were detected in influent and 4 in effluent.

In influent, the highest concentration detected was for sucralose (32 µg/L), and of the 4 detected artificial sweeteners, sucralose had the highest concentration consistently at each of the WWTP sites. The other artificial sweeteners detected in influent were acesulfame K, saccharin and cyclamate. All 4 of these artificial sweeteners were detected in influent at all WWTP sites (100% detection). Across WWTP sites, the mean concentrations of artificial sweeteners in influent from highest to lowest were, as follows: Site C > Site D > Site B > Site A, with the highest maximum concentration in influent observed at Site D (Table 3).

Similarly, the highest concentration detected in effluent was sucralose (34 µg/L), with the other detected artificial sweeteners being acesulfame K, saccharin, and cyclamate. As with influent, all 4 of these artificial sweeteners were also detected in effluent at all the WWTP sites (100% detection). Across WWTP sites, the mean concentrations of artificial sweeteners in effluent from highest to lowest were as follows: Site D > Site A > Site C > Site B, with the highest maximum concentration in effluent observed at Site D (Table 3).

4.1.3. Endocrine disrupting chemicals (EDCs)

For EDCs, concentrations in influent ranged from <0.002 µg/L to 23 µg/L, and from <0.002 µg/L to 24 µg/L in effluent (Table 2). Of the 15 EDCs analysed in wastewater, 10 were detected in influent and 6 in effluent.

In influent, the highest concentrations of EDCs were for nonylphenol (23 µg/L), butylated hydroxytoluene (BHT; 1.7 µg/L), etiocholanolone (1.3 µg/L) and androsterone (0.55 µg/L). The most frequently detected compounds in influent were etiocholanolone, estrone, estriol, and bisphenol A (100% detection across all 4 WWTP sites), followed by testosterone (75%). EDCs detected only in influent and not in effluent included androsterone, estriol, etiocholanolone, and testosterone. Across WWTP sites, the mean concentrations of EDCs in influent from highest to lowest were, as follows: Site C > Site A > Site B > Site D, with the highest maximum concentration in influent observed at Site C (Table 3).

In effluent, the highest concentrations of EDCs were for nonylphenol (24 µg/L), BHT (0.54 µg/L), and tert-octyl phenol (0.42 µg/L). The most frequently detected compounds in effluent were estrone and bisphenol A (100%), followed by nonylphenol and tert-octyl phenol (50%). Across WWTP sites, the mean concentrations of EDCs in effluent from highest to lowest were, as follows: Site C > Site A > Site D > Site B, with the highest maximum concentration in effluent observed at Site C (Table 3).

4.1.4. Per- and polyfluoroalkyl substances (PFAS)

Concentrations of PFAS ranged from <0.0002 µg/L to 0.41 µg/L in influent, and <0.0002 µg/L to 0.44 µg/L in effluent (Table 2). Of the 41 PFAS analysed in wastewater, 17 were detected in influent, and 16 in effluent.

In influent, the highest PFAS concentration detected was for PFOS (0.41 µg/L), followed by PFHxS (0.35 µg/L), PFHxA (0.095 µg/L), PFBS (0.048 µg/L), PFPeS (0.046 µg/L), PFPeA (0.044 µg/L), and 5:3 FTCA (0.043 µg/L). Furthermore, PFOS, PFHxS, PFBS, PFPeA, PFOA, PFHxA, PFHpA, 6:2 FTS, 5:3 FTCA were detected in 100% of WWTP influent samples. PFAS that was detected only in influent but not in effluent was 5:3 FTCA. Across WWTP sites, the mean concentrations of PFAS in influent from highest to lowest were, as follows: Site A > Site C > Site D > Site B, with the highest maximum concentration in influent observed at Site A (Table 3).

In effluent, highest concentrations for PFAS were detected for PFHxS (0.44 µg/L), PFOS (0.3 µg/L), PFHxA (0.14 µg/L), PFPeA (0.073 µg/L), PFBS (0.069 µg/L), PFPeS (0.061 µg/L), and PFOA (0.033 µg/L). Furthermore, PFOS, PFHxS, PFBS, PFPeA, PFOA, PFHxA, and PFHpA, were detected in 100% of WWTP effluent samples. Across WWTP sites, the mean concentrations of PFAS in effluent from highest to lowest were, as follows: Site A > Site C > Site B > Site D, with the highest maximum concentration in effluent observed at Site A (Table 3).

4.1.5. Pesticides

Concentrations of pesticides in influent ranged from <0.01 µg/L to 3.2 µg/L in influent, and from <0.01 µg/L to 0.36 µg/L in effluent (Table 2). Of the 442 pesticides analysed in wastewater, 25 were detected in influent, and 19 were detected in effluent.

In influent, the highest concentrations of pesticides were for DEET (3.2 µg/L), propham (0.45 µg/L), prothioconazole (0.3 µg/L), piperonyl butoxide (0.25 µg/L), and cyromazine (0.14 µg/L). The most frequently detected pesticides in influent were DEET, diuron, imidacloprid, permethrin, and piperonyl butoxide (100%), followed by MGK-264, metsulfuron-methyl, propiconazole, spirotetramat-enol, and tebuconazole (75%). Pesticides that were detected only in influent and not in effluent were azoxystrobin, benalaxyl, diazinon, DMST, MGK-264, permethrin, piperonyl butoxide, propham and propoxur. Across WWTP sites, the mean concentrations of pesticides in influent from highest to lowest were, as follows: Site C > Site B > Site A > Site D, with the highest maximum concentration in influent observed at Site C (Table 3).

In effluent, the highest concentrations of pesticides detected were DEET (0.36 µg/L), prothioconazole (0.12 µg/L), diuron (0.11 µg/L), and imidacloprid (0.08 µg/L). The most frequently detected pesticides in

effluent were DEET, diuron and imidacloprid (100%), followed by tebuconazole (75%). Pesticides detected in effluent that were not detected in influent included epoxiconazole, metazachlor, and methamidophos. Across WWTP sites, the mean concentrations of pesticides in effluent from highest to lowest were, as follows: Site A > Site C > Site D > Site B, with the highest maximum concentration in effluent observed at Site C (Table 3).

4.1.6. Phthalates

For phthalates, concentrations in influent ranged from <0.01 µg/L to 64 µg/L, and from <0.01 µg/L to 7.6 µg/L in effluent (Table 2). Of the 19 phthalates analysed in wastewater, 6 were detected in influent, and 4 were detected in effluent.

In influent, the highest concentrations of phthalates were for di-n-pentyl phthalate (DnPP; 64 µg/L), di-ethylhexyl phthalate (DEHP; 3.8 µg/L), and diethyl phthalate (DEP; 1.2 µg/L). The most frequently detected phthalate in influent was DEHP (100%), followed by DEP (75%) and di-isobutyl phthalate (DIBP; 75%), then DnPP (50%) and dimethyl phthalate (DMP; 50%), then di-n-butyl phthalate (DBP; 25%). DBP and DnPP were detected only in influent samples, and not in effluent samples. Across WWTP sites, the mean concentrations of phthalates in influent from highest to lowest were, as follows: Site B > Site D > Site C > Site A, with the highest maximum concentration in influent observed at Site B (Table 3).

In effluent, the highest concentrations of phthalates were for DEHP (7.6 µg/L), followed by DEP (0.25 µg/L), then DIBP (0.05 µg/L). The most frequently detected phthalates in effluent were DEHP and DEP (50%), then DIBP and DMP (25%). All phthalates that were detected in effluent were also detected in influent. Across WWTP sites, the mean concentrations of phthalates in effluent from highest to lowest were, as follows: Site C > Site D > Site A > Site B, with the highest maximum concentration in effluent observed at Site C (Table 3).

Table 2. Summary statistics of emerging contaminants detected at each sampling point (wastewater and surface water) for each analyte group. EDCs = endocrine disrupting chemicals, PFAS = per- and polyfluoroalkyl substances, PPCPs = pharmaceuticals and personal care products. nd = not detected. LOR = limit of reporting.

Analyte group	Sampling point	Chemicals analysed (*n*)	Chemicals detected (*n*)	Mean (µg/L)	SD (µg/L)	Min (µg/L)	Max (µg/L)	LOR (µg/L)
Artificial Sweeteners	Upstream	11	4	0.015	0.059	nd	0.37	0.005
	Influent	11	4	3.48	7.79	nd	32	0.005
	Effluent	11	4	2.60	8.25	nd	34	0.005
	Discharge	11	4	0.29	1.35	nd	7.2	0.005
	Downstream	11	4	0.32	1.54	nd	8.8	0.005
	Further downstream	11	4	0.19	0.97	nd	6.7	0.005
EDCs	Upstream	15	0	0	0	nd	nd	0.001–0.05
	Influent	15	10	0.53	2.97	nd	23	0.001–0.05
	Effluent	15	6	0.43	3.10	nd	24	0.001–0.05
	Discharge	15	3	0.065	0.55	nd	4.8	0.001–0.05
	Downstream	15	2	0.016	0.14	nd	1.2	0.001–0.05
	Further downstream	15	2	0.0072	0.062	nd	0.54	0.001–0.05
PFAS	Upstream	46	13	0.0011	0.0054	nd	0.048	0.0002–0.0005
	Influent	41	17	0.011	0.053	nd	0.41	0.0002–0.0005
	Effluent	41	16	0.012	0.056	nd	0.44	0.0002–0.0005
	Discharge	46	14	0.0013	0.0053	nd	0.033	0.0002–0.0005
	Downstream	46	13	0.0016	0.0084	nd	0.097	0.0002–0.0005
	Further downstream	46	13	0.00092	0.0040	nd	0.039	0.0002–0.0005
Pesticides	Upstream	445	13	0.00018	0.0029	nd	0.11	0.01
	Influent	442	25	0.0057	0.10	nd	3.2	0.01
	Effluent	442	19	0.00095	0.012	nd	0.36	0.01
	Discharge	445	12	0.00027	0.0036	nd	0.1	0.01
	Downstream	445	11	0.00019	0.0027	nd	0.09	0.01
	Further downstream	445	10	0.00014	0.0023	nd	0.09	0.01
PPCPs	Upstream	101	6	0.0012	0.0098	nd	0.17	0.005
	Influent	101	62	2.45	14.30	nd	200	0.005
	Effluent	101	51	0.16	0.74	nd	12	0.005
	Discharge	101	33	0.010	0.051	nd	0.54	0.005
	Downstream	101	32	0.011	0.064	nd	0.99	0.005
	Further downstream	101	21	0.0034	0.021	nd	0.26	0.005
PPCPs (illicit)	Upstream	8	0	0	0	nd	nd	0.005
	Influent	8	5	0.24	0.45	nd	1.8	0.005
	Effluent	8	4	0.035	0.088	nd	0.35	0.005
	Discharge	8	2	0.0011	0.0048	nd	0.024	0.005
	Downstream	8	2	0.0012	0.0051	nd	0.029	0.005

	Further downstream	8	1	0.00020	0.0013	nd	0.008	0.005
Phthalates	Upstream	19	4	0.0059	0.029	nd	0.23	0.01
	Influent	19	6	1.08	7.37	nd	64	0.01
	Effluent	19	4	0.10	0.87	nd	7.6	0.01
	Discharge	19	4	0.0034	0.020	nd	0.18	0.01
	Downstream	19	4	0.0048	0.029	nd	0.24	0.01
	Further downstream	19	4	0.0055	0.026	nd	0.18	0.01

Table 3. Site-specific summary statistics of emerging contaminants detected in wastewater (influent and effluent) at each WWTP (Sites A–D) and across each analyte group. EDCs = endocrine disrupting chemicals, PFAS = per- and polyfluoroalkyl substances, PPCPs = pharmaceuticals and personal care products. nd = not detected. LOR = limit of reporting

Analyte group	Sampling site	Sampling point	Chemicals analysed (*n*)	Chemicals detected (*n*)	Mean (µg/L)	SD (µg/L)	Min (µg/L)	Max (µg/L)	LOR (µg/L)
Artificial Sweeteners	Site A	Influent	11	4	2.33	5.16	nd	17	0.005
	Site B	Influent	11	4	3.24	7.54	nd	25	0.005
	Site C	Influent	11	4	5.05	9.03	nd	25	0.005
	Site D	Influent	11	4	3.30	9.55	nd	32	0.005
	Site A	Effluent	11	4	2.85	9.01	nd	30	0.005
	Site B	Effluent	11	4	1.92	6.33	nd	21	0.005
	Site C	Effluent	11	4	2.52	8.12	nd	27	0.005
	Site D	Effluent	11	4	3.11	10.25	nd	34	0.005
EDCs	Site A	Influent	15	10	0.25	0.45	nd	1.3	0.001–0.05
	Site B	Influent	15	5	0.041	0.090	nd	0.33	0.001–0.05
	Site C	Influent	15	9	1.80	5.89	nd	23	0.001–0.05
	Site D	Influent	15	5	0.036	0.066	nd	0.21	0.001–0.05
	Site A	Effluent	15	4	0.045	0.13	nd	0.5	0.001–0.05
	Site B	Effluent	15	2	0.0013	0.0038	nd	0.014	0.001–0.05
	Site C	Effluent	15	6	1.67	6.18	nd	24	0.001–0.05
	Site D	Effluent	15	2	0.0019	0.0058	nd	0.022	0.001–0.05
PFAS	Site A	Influent	41	17	0.041	0.10	nd	0.41	0.0002–0.0005
	Site B	Influent	41	11	0.00026	0.00054	nd	0.002	0.0002–0.0005
	Site C	Influent	41	15	0.0022	0.0050	nd	0.02	0.0002–0.0005
	Site D	Influent	41	11	0.00048	0.0011	nd	0.0049	0.0002–0.0005
	Site A	Effluent	41	16	0.043	0.11	nd	0.44	0.0002–0.0005
	Site B	Effluent	41	10	0.00065	0.0026	nd	0.016	0.0002–0.0005
	Site C	Effluent	41	13	0.0023	0.0064	nd	0.037	0.0002–0.0005
	Site D	Effluent	41	10	0.00038	0.0012	nd	0.0068	0.0002–0.0005
Pesticides	Site A	Influent	442	18	0.0051	0.061	nd	1.2	0.01
	Site B	Influent	442	11	0.0056	0.10	nd	2.2	0.01
	Site C	Influent	442	15	0.0091	0.15	nd	3.2	0.01
	Site D	Influent	442	10	0.0031	0.052	nd	1.1	0.01
	Site A	Effluent	442	15	0.0017	0.014	nd	0.24	0.01
	Site B	Effluent	442	5	0.00029	0.0033	nd	0.06	0.01
	Site C	Effluent	442	9	0.0014	0.018	nd	0.36	0.01
	Site D	Effluent	442	5	0.00040	0.0045	nd	0.08	0.01
PPCPs	Site A	Influent	101	47	1.37	5.32	nd	43	0.005
	Site B	Influent	101	56	4.02	22.25	nd	200	0.005
	Site C	Influent	101	56	3.06	13.25	nd	102	0.005
	Site D	Influent	101	35	1.34	10.96	nd	110	0.005
	Site A	Effluent	101	41	0.28	0.79	nd	6.4	0.005
	Site B	Effluent	101	41	0.19	1.20	nd	12	0.005
	Site C	Effluent	101	43	0.075	0.17	nd	0.96	0.005

	Site D	Effluent	101	38	0.087	0.30	nd	2.3	0.005
PPCPs (illicit)	Site A	Influent	8	4	0.21	0.39	nd	1.1	0.005
	Site B	Influent	8	5	0.18	0.26	nd	0.77	0.005
	Site C	Influent	8	3	0.27	0.63	nd	1.8	0.005
	Site D	Influent	8	5	0.31	0.50	nd	1.4	0.005
	Site A	Effluent	8	4	0.11	0.15	nd	0.35	0.005
	Site B	Effluent	8	1	0.0030	0.0085	nd	0.024	0.005
	Site C	Effluent	8	3	0.0090	0.014	nd	0.035	0.005
	Site D	Effluent	8	3	0.021	0.045	nd	0.13	0.005
Phthalates	Site A	Influent	19	4	0.095	0.29	nd	1.2	0.01
	Site B	Influent	19	4	3.57	14.65	nd	64	0.01
	Site C	Influent	19	5	0.28	0.90	nd	3.8	0.01
	Site D	Influent	19	2	0.37	1.40	nd	6.1	0.01
	Site A	Effluent	19	1	0.00053	0.0023	nd	0.01	0.01
	Site B	Effluent	19	0	0	0	nd	nd	0.01
	Site C	Effluent	19	4	0.42	1.74	nd	7.6	0.01
	Site D	Effluent	19	1	0.0011	0.0046	nd	0.02	0.01

4.2. Estimated percent reduction (%R) of emerging contaminants

The estimated percent reduction (%R) of emerging contaminants across the four WWTP sites (Sites A–D) demonstrated a wide range of reduction, with near complete reduction (>99.9 %R) for some chemicals, to an increase by up to 700% (i.e., -700 %R) in effluent concentration compared to influent.

4.2.1. PPCPs

Mean %R for individual PPCPs were relatively high overall, except for compounds which had a negative mean %R (see Appendix B, Table S2). Across all WWTP sites, in general, %R ranged from -450% to >99.9%, with an overall mean %R of 55.72% (standard deviation (SD) = 82.99%). Between sites, mean %R was highest at Site B (TT7, 80.41%), followed by Site C (TT7, 76.59%), Site D (TT14, 28.67%), then Site A (TT4, 21.57%). Comparing to the 2021 EPA study (EPA, 2023), %R estimates for PPCPs across treatment trains were broadly similar, with high reduction seen in compounds such as paracetamol and caffeine, but low to negative %R seen for compounds such as lamotrigine and carbamazepine across the same treatment train types.

4.2.2. Artificial sweeteners

%R for artificial sweeteners was found to be compound specific. Specifically, 3 out of 4 of the artificial sweeteners detected (acesulfame K, cyclamate, saccharin) had a mean %R of 90.05% to 95.85%, but sucralose had a poor mean %R of -18.68% (i.e. an increase in mean concentration of 18.68% in effluent compared to influent). In general, across all WWTP sites, %R ranged from -76.5% up to 98.74%, with an overall mean %R of 64.87% (SD = 53.45%). Between sites, mean %R for artificial sweeteners was highest at Site B (TT7, 77.22%), followed by Site C (TT7, 70.99%) and Site D (TT14, 70.02%), and lowest at Site A (TT4, 41.23%). Artificial sweeteners as a group were not targeted in the 2021 study (EPA Publication 2054; EPA, 2023). However, acesulfame K was previously included as a PPCP (EPA, 2023), and %R values were found to be relatively consistent between this study and the 2021 study for the same treatment train types (TT4, 7, 8, 14).

4.2.3. EDCs

%R for EDCs varied between compounds, with mean %R ranging for individual compounds from -80.91% up to near complete removal of >99.9% (see Appendix B, Table S2). For EDCs, across all WWTP sites, %R ranged from -164.15% to >99.9%, with an overall mean %R of 64.56% (SD = 60.87%). Between sites, mean %R for EDCs was highest at Site B (TT7, 76.22%), followed by Site D (TT14, 72.86%), Site C (TT7, 65.04%), then Site A (TT4, 54.16%). Mean %R for androsterone, BPA, estriol, and etiocholanolone were similar to results from the 2021 study with high removal efficiencies across all treatment trains (EPA, 2023). However, the %R for nonylphenol and estrone differed from the 2021 study. The concentrations found in this study indicated a %R of -40% (Site A), and -20% (Site D) for estrone, and -4.35% for nonylphenol (Site C), which was much lower than the %R of above 66% for both chemicals across all treatment trains in the previous study (EPA, 2023). It is however important to note that the low %R for nonylphenol and estrone observed in this study was for a singular sample at each specific site. Additional factors such as mixing pond detention time, the volume of influent, and the scale of each treatment process between sites may play a role in the differences seen between treatment trains for this and the previous 2021 study.

4.2.4. PFAS

Interestingly, PFAS was the only chemical group with a negative mean %R, indicating that on average, compound concentrations increased from influent to effluent with overall poor removal efficiencies. Across all WWTP sites, %R for PFAS ranged from -700% to >99.9%, with an overall mean %R of -35.39% (SD = 160.88%). For individual PFAS, negative mean %R estimates were observed for more than half of the detected compounds (see Appendix B, Table S2). Between sites, mean %R for PFAS was highest at Site D (TT14, -8.89%), followed by Site C (TT7, -11.80%) then Site A (TT4, -12.70%), then Site B (TT7, -129.14%). The poor %R for PFAS across the sampled treatment trains here is broadly consistent with findings for the same treatment train types (TT4, 7, 8, 14) previously studied (EPA, 2023). The low removal efficacy of PFAS due to high resistance to degradation and increase of certain PFAS compounds following wastewater treatment has been shown to be a typical finding in other studies as well (reviewed in Lenka et al., 2021).

4.2.5. Pesticides

Most pesticides had relatively high estimated removal efficiencies, with 10 out of the 26 detected compounds approaching total estimated removal from influent (>99.9%). Across all WWTP sites, %R ranged from -100% to >99.9%, with an overall mean %R of 57.53% (SD = 57.99%). For individual pesticides, a negative mean %R was reported for several compounds, which included thiabendazole, diuron, and imidacloprid (see Appendix B, Table S2). Between sites, mean %R was highest at Site D (TT14, 83.92%), then Site B (TT7, 73.51%), Site C (TT7, 56.92%), and Site A (TT4, 33.61%). When compared to previous results, we see broadly similar findings in percent reduction estimates across treatment trains, that is, relatively high %R for pesticides overall, but with compound specificity (EPA, 2023). For example, diuron has been shown to have low to negative %R across the treatment trains sampled in both the previous and current study.

4.2.6. Phthalates

Mean %R for individual phthalates were all positive and relatively high (see Appendix B, Table S2). Across all WWTP sites, %R of phthalates ranged from -100% to >99.9%, with an overall mean %R of 81.59% (SD = 51.30%). %R for phthalates approached total removal for Site B (TT7, >99.9%), Site D (TT14, >99.9%), and Site A (TT4, 99.79%), but not Site C (TT7, 44.95%). The low mean %R observed at Site C was primarily driven by a 100% increase (i.e. -100 %R) in the concentration of DEHP from influent to effluent, with positive %R for other phthalates at this site. Phthalates were not investigated in the previous 2021 EPA study (EPA, 2023), and so more data is needed for comparison.

4.3. Differences between the receiving surface water environment across WWTP sites

4.3.1. PPCPs

For PPCPs, on average, a substantial increase was observed in mean surface water concentrations from the upstream location (mean ± SD: 0.0012 ± 0.0098 µg/L) to the discharge point (0.010 ± 0.051 µg/L) and downstream location (0.011 ± 0.064 µg/L) (Figure 2). Mean PPCP concentration was also higher in the second downstream location (0.0034 ± 0.021 µg/L) compared to upstream of the discharge (Fig. 2). These trends indicate that effluent-discharge may be one of the predominant sources of PPCPs into the environment. However, low concentrations of several PPCPs were also detected at both reference sites F and G, as evidence of the ubiquitous and pervasive nature of these compounds (Figure 3).

PPCPs were detected in the surface water environment across all effluent-receiving sites (Sites A–E), but with varying trends between sampling locations (Figure 3). Although a large number of PPCPs were detected in influent and effluent at all sampled WWTP sites (Sites A–D), there was no clear increase in PPCP concentrations from upstream to downstream locations at Sites A, B and D, with only one PPCP detected (nicotine) in all surface water samples at Site B and only one PPCP detected (gabapentin) in the downstream location at Site D (Figure 3). However, at Sites C and E, we observed a clear increase in the concentrations of PPCPs from upstream to the discharge and downstream locations (Figure 3).

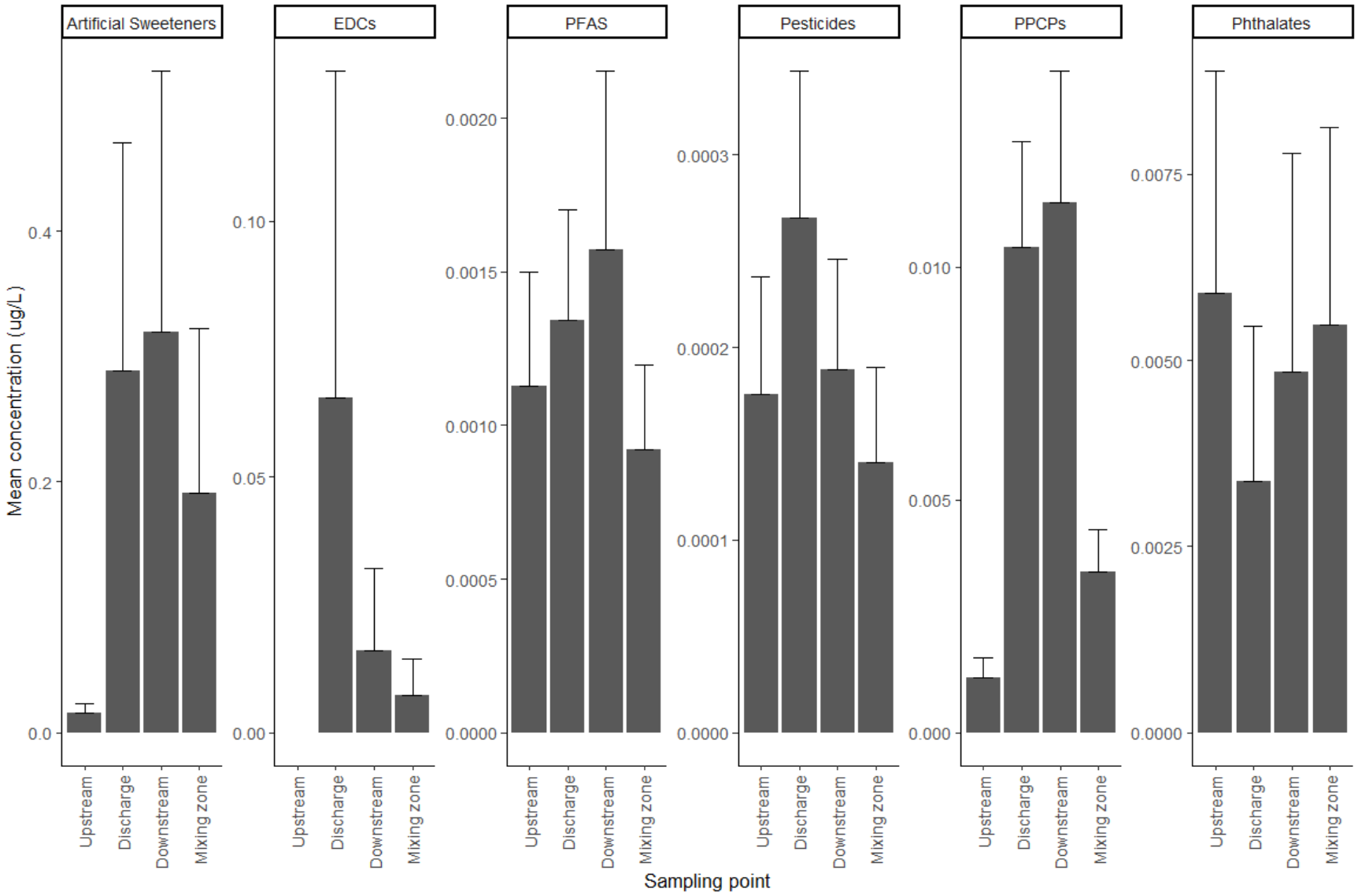

Figure 2. Mean concentrations (µg/L) of emerging contaminants detected for each analyte group at each surface water sampling point, including standard error bars. EDCs = endocrine disrupting chemicals, PFAS = per- and polyfluoroalkyl substances, PPCPs = pharmaceuticals and personal care products.

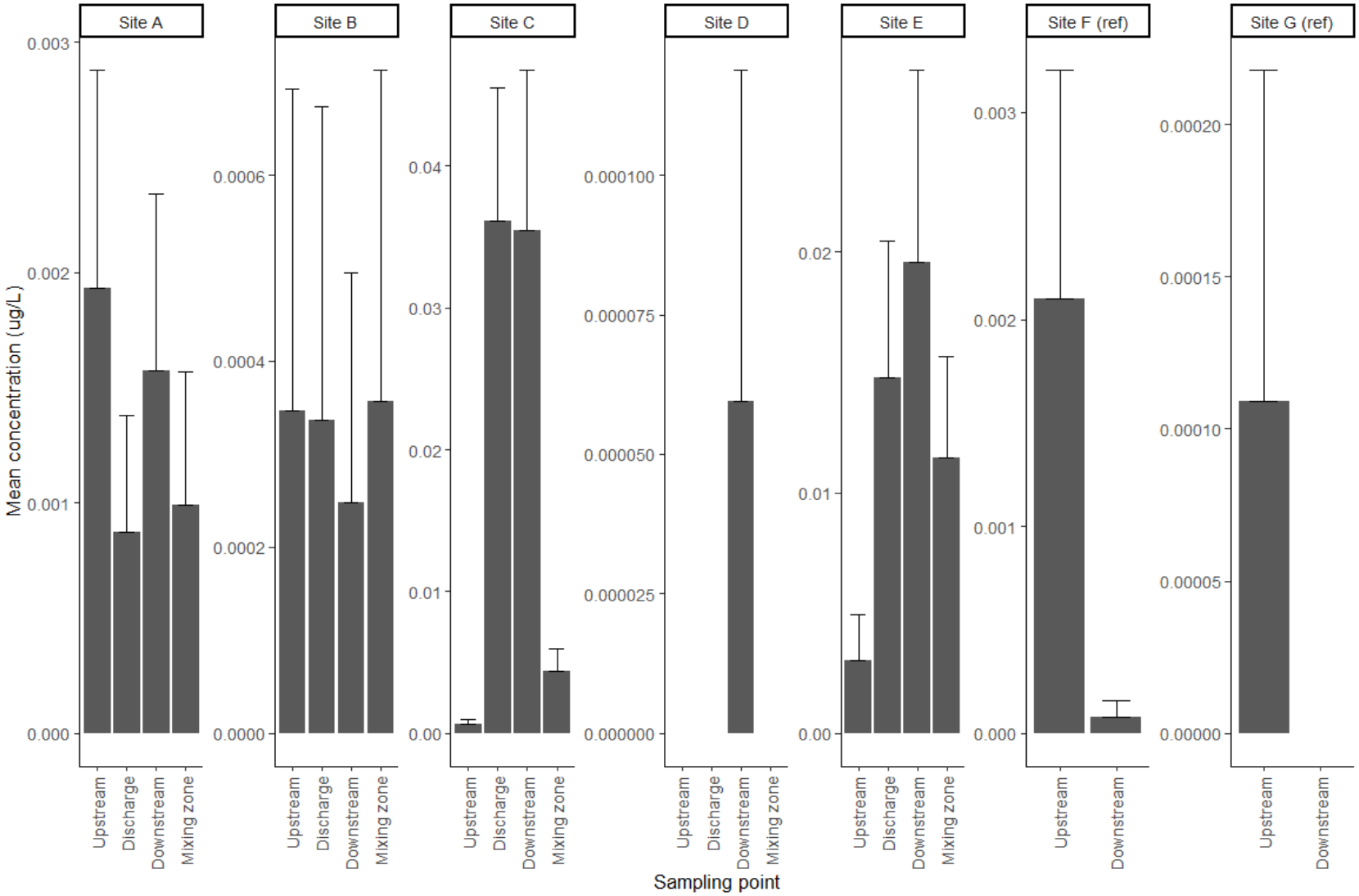

Figure 3. Mean concentrations (µg/L) of pharmaceuticals and personal care products (PPCPs) detected at each surface water sampling point separated by site (Sites A–F), including standard error bars.

4.3.2. Artificial sweeteners

On average, in the effluent-receiving surface water environments (Sites A–E), a marked increase was observed in the mean concentrations of artificial sweeteners from upstream of the effluent discharge (mean ± SD: 0.015 ± 0.059 μg/L) to the effluent discharge point (0.29 ± 1.35 μg/L) and downstream of the discharge (0.32 ± 1.54 μg/L) (Figure 2). Furthermore, there were still higher mean levels (approx. 10×) of artificial sweeteners in the further downstream location (0.19 ± 0.97 μg/L) compared to upstream of the discharge. Together, these results indicate that effluent discharge may be one of the predominant sources of artificial sweeteners into the environment. However, one artificial sweetener (sucralose) was also detected at relatively low mean concentrations at one of the two reference sites, Site F (Figure 4).

When comparing between the five effluent-receiving surface water sites (Sites A–E), there was a variation in the trends of artificial sweetener input into the environment across sites. Specifically, at Sites A and B, there were no major differences in mean surface water concentrations of artificial sweeteners from the upstream location compared to the discharge and downstream locations, even though artificial sweeteners were detected in effluent at relatively high concentrations (Figure 4). At Sites C, D and E, however, there was an increase in mean concentrations of artificial sweeteners at the discharge and downstream locations compared to the upstream location (Figure 4), indicating input into surface water via effluent-discharge at these sites.

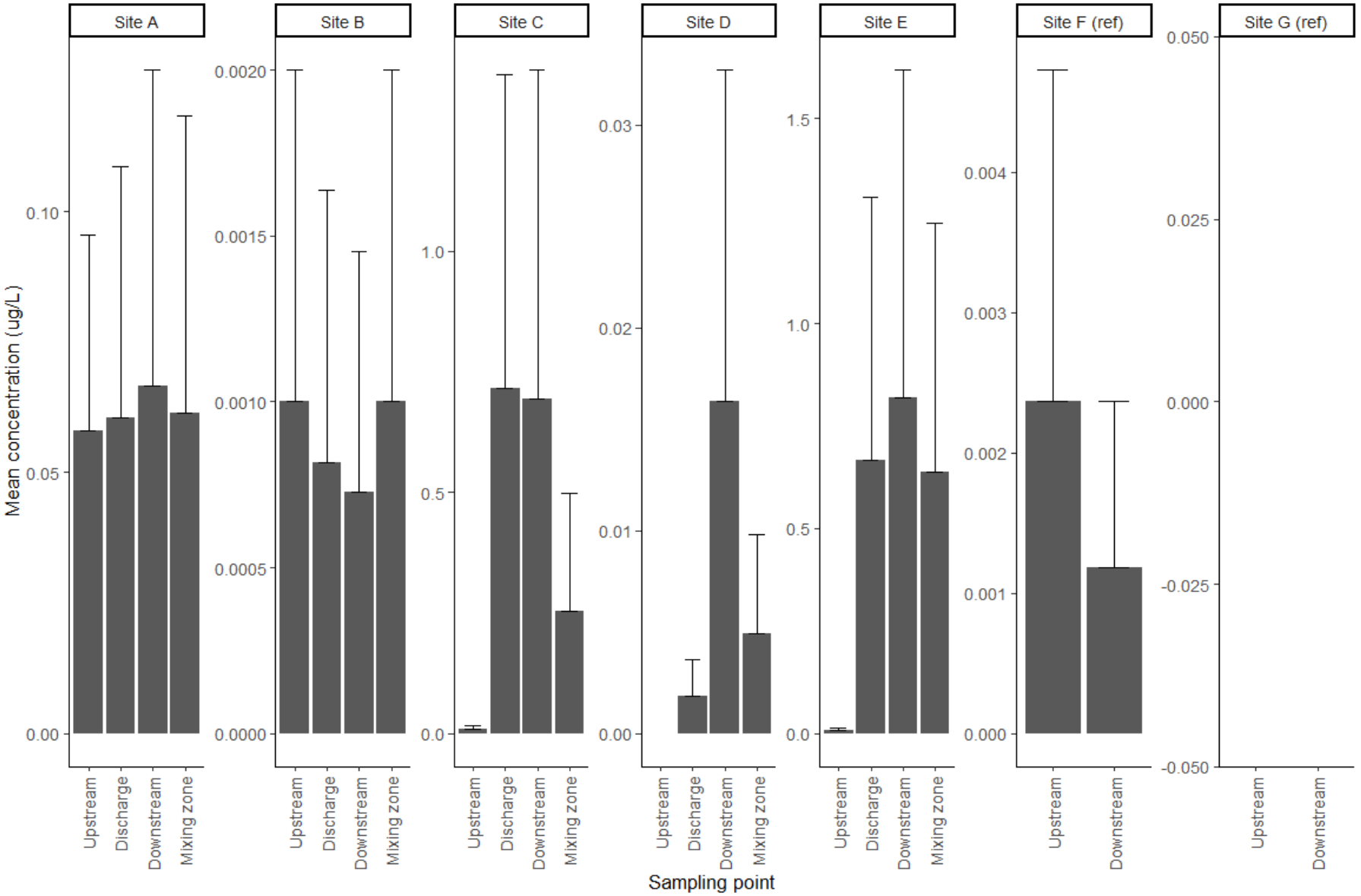

Figure 4. Mean concentrations (µg/L) of artificial sweeteners detected at each surface water sampling point separated by site (Sites A–F), including standard error bars.

4.3.3. EDCs

We observed addition of EDCs into the surface water environment from effluent discharge (Sites A–E) only, with no EDCs detected upstream of effluent discharge at any of the sites. EDCs were detected at the discharge point (mean ± SD: 0.065 ± 0.55 μg/L), and at the first downstream (0.016 ± 0.14 μg/L) and second downstream locations (0.0072 ± 0.062 μg/L) (Figure 2). Additionally, EDCs were not detected at either of the reference sites (Fig. 5).

Interestingly, although EDCs were detected in wastewater influent and effluent at all WWTP sites sampled (Sites A–D), when comparing between the five effluent-receiving surface water sites (Sites A–E), EDCs were detected in surface water at only two of the sites (Site C & E), and not at Sites A, B or D (Figure 5). The overall trends of EDCs in surface water appear to be driven primarily by higher loads of EDCs detected in the discharge and downstream locations at Site C (Figure 5).

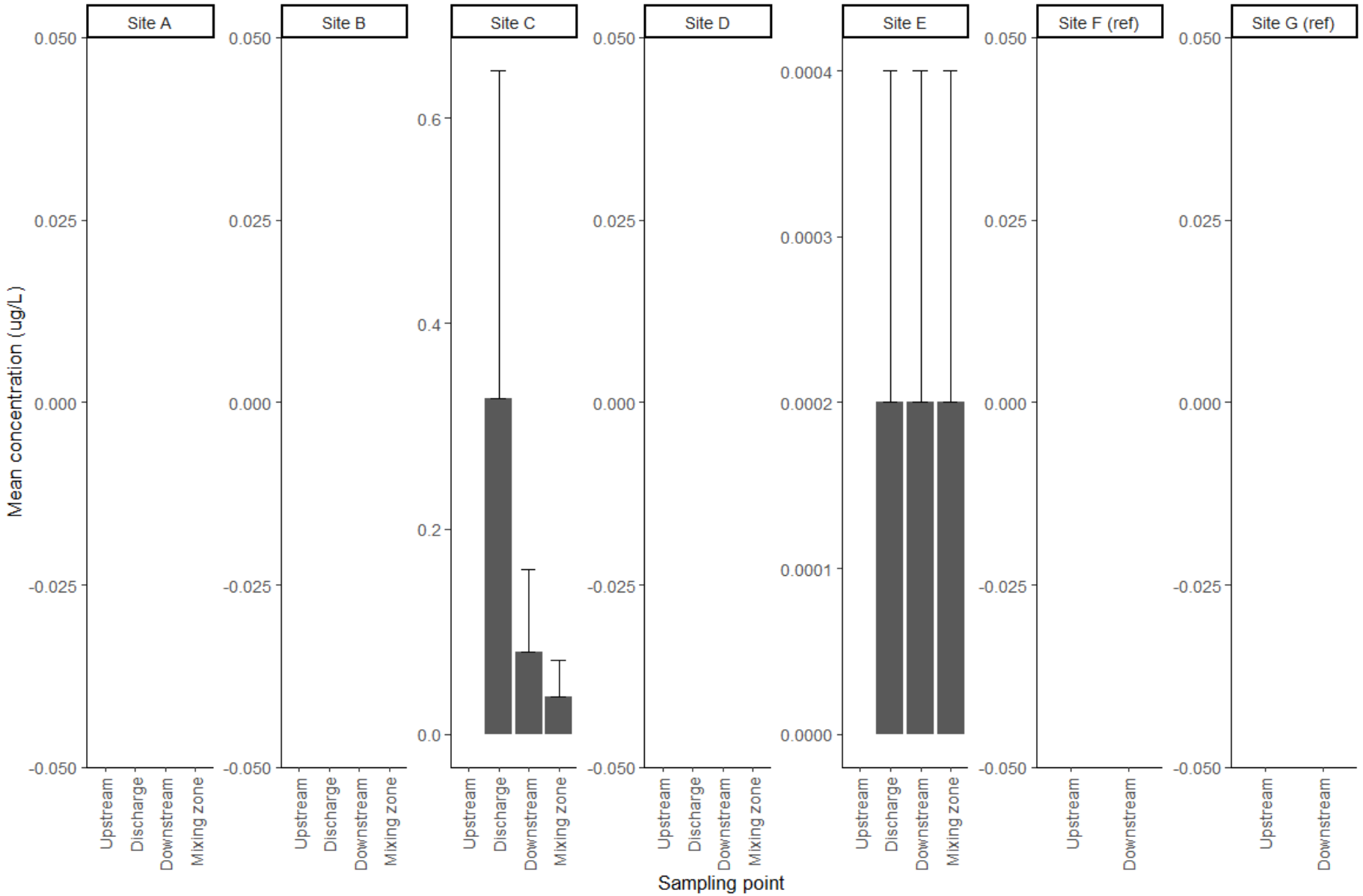

Figure 5. Mean concentrations (µg/L) of endocrine disrupting chemicals (EDCs) detected at each surface water sampling point separated by site (Sites A–F), including standard error bars.

4.3.4. PFAS

For PFAS, on average across all sites, there was a slight increase in mean surface water concentrations from upstream of the discharge point (mean ± SD: 0.0011 ± 0.0054 µg/L), to the discharge point (0.0013 ± 0.0053 µg/L) and the first downstream location (0.0016 ± 0.0084 µg/L), with a slight decrease at the second downstream location (0.0009 ± 0.0040 µg/L) (Figure 2). These results indicate that effluent discharge is only one of the sources of PFAS into the environment. Diffuse non-point sources, such as stormwater, also contribute to PFAS loads in surface water environments (Saifur and Gardner, 2021; Zushi et al., 2008). PFAS were not detected at either of the reference sites (Figure 6).

Our results indicate that sources of PFAS into the surface water environment are site-specific and input sources may vary. For example, at Site A, PFAS concentrations did not differ significantly between upstream, discharge and downstream locations (Figure 6). However, at Sites C and E, an increase in PFAS concentrations was observed at discharge and downstream locations compared to the upstream location (Figure 6).

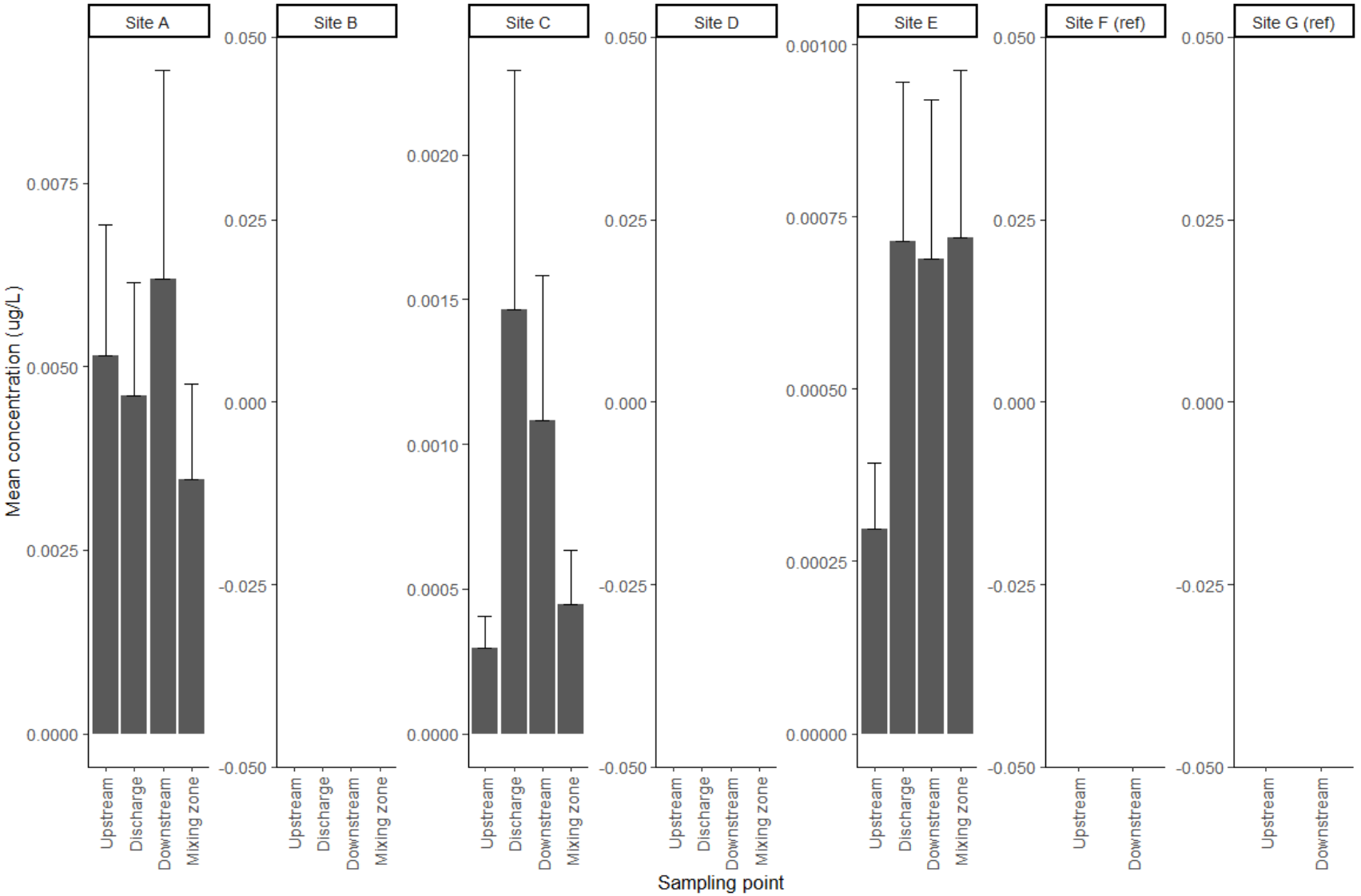

Figure 6. Mean concentrations (µg/L) of per- and polyfluoroalkyl substances (PFAS) detected at each surface water sampling point separated by site (Sites A–F), including standard error bars.

4.3.5. Pesticides

On average, we see an increase in mean surface water concentrations of pesticides from the upstream location (mean ± SD: 0.00018 ± 0.0029 μg/L) to the discharge point (0.00027 ± 0.0036 μg/L). However, mean pesticide concentrations at the first downstream (0.00019 ± 0.0027 μg/L) and second downstream locations (0.00014 ± 0.0023 μg/L) were similar to concentrations observed upstream (Figure 2). Additionally, pesticides were not detected in surface water at either of the reference sites (Figure 7).

Across the effluent-receiving surface water sites, pesticides were only detected in surface water at Sites A, C, and E (Figure 7). An increase in pesticide concentrations was observed from the upstream to downstream locations in surface water at Site C, but pesticide loads in surface water were stable or decreased from upstream to downstream locations at Site A and E (Figure 7). These results again highlight the site-specificity in environmental sources of contaminants such as pesticides, with varying degrees of contribution from effluent discharges to total pesticide loads in surface water.

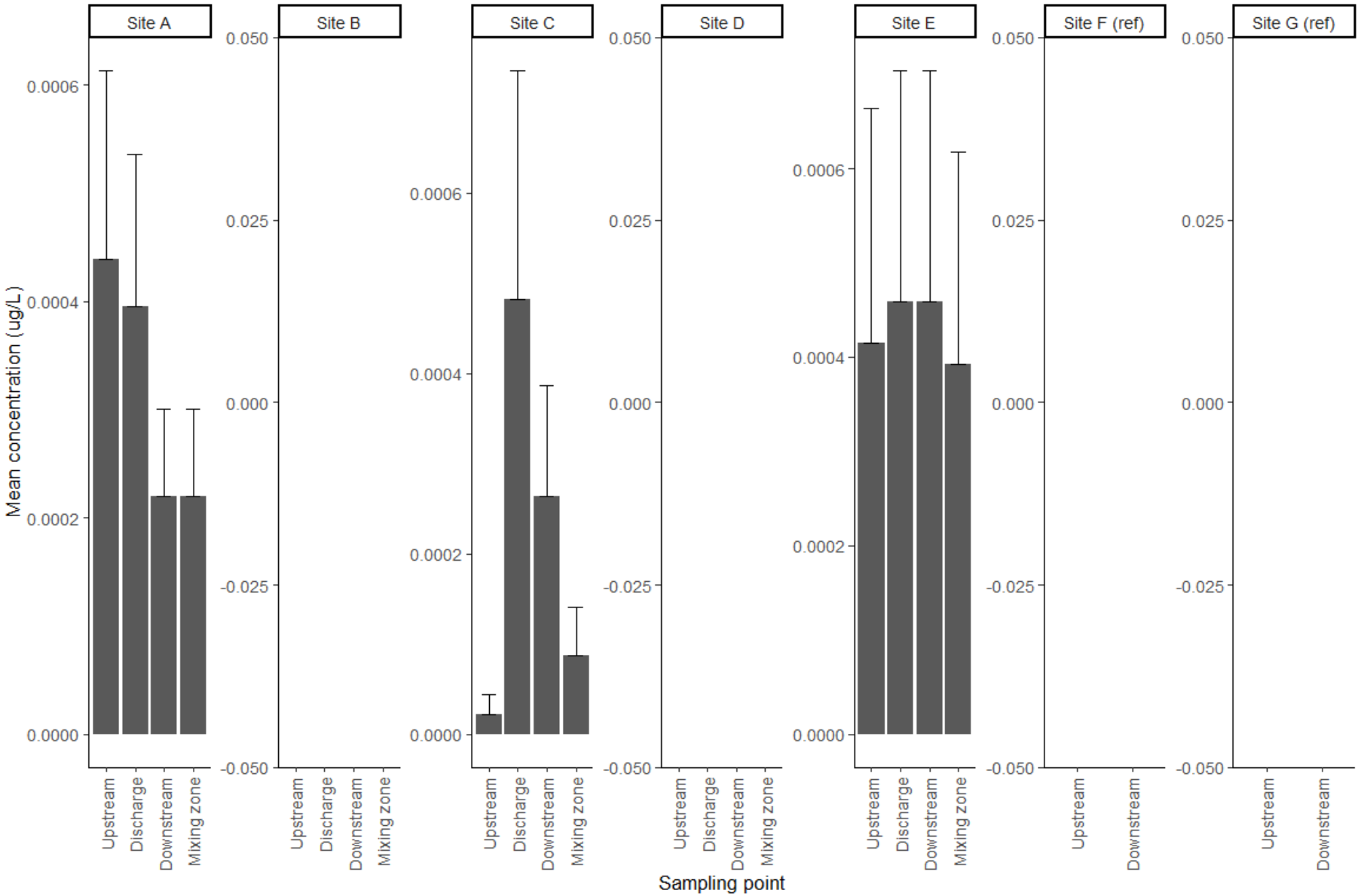

Figure 7. Mean concentrations (µg/L) of pesticides detected at each surface water sampling point separated by site (Sites A–F), including standard error bars.

4.3.6. Phthalates

There was no clear trend in the surface water concentrations of phthalates from upstream to downstream locations (Fig. 2). On average, the highest mean phthalate concentration was observed at the upstream location (mean ± SD: 0.0059 ± 0.029 μg/L), followed by the second further downstream location, (0.0055 ± 0.026 μg/L), then the first downstream location (0.0048 ± 0.029 μg/L), and the discharge point (0.0034 ± 0.020 μg/L) (Fig. 2). No phthalates were detected at either of the reference sites (Fig. 8).

When comparing between the effluent-receiving surface water sites (Sites A–E), again, no clear trend was observed for the input of phthalates via effluent discharge as a primary environmental contributor at any of the sites (Fig. 8). Specifically, phthalates were not detected in surface water at Sites B and D, the upstream location had the highest phthalate concentrations at Sites A and C, and there was no clear increasing trend of phthalates along the waterway at Site E (Fig. 8). These results indicate that, unlike the other contaminant groups, effluent discharge does not appear to be the primary source of phthalate loads in the surface water environment.

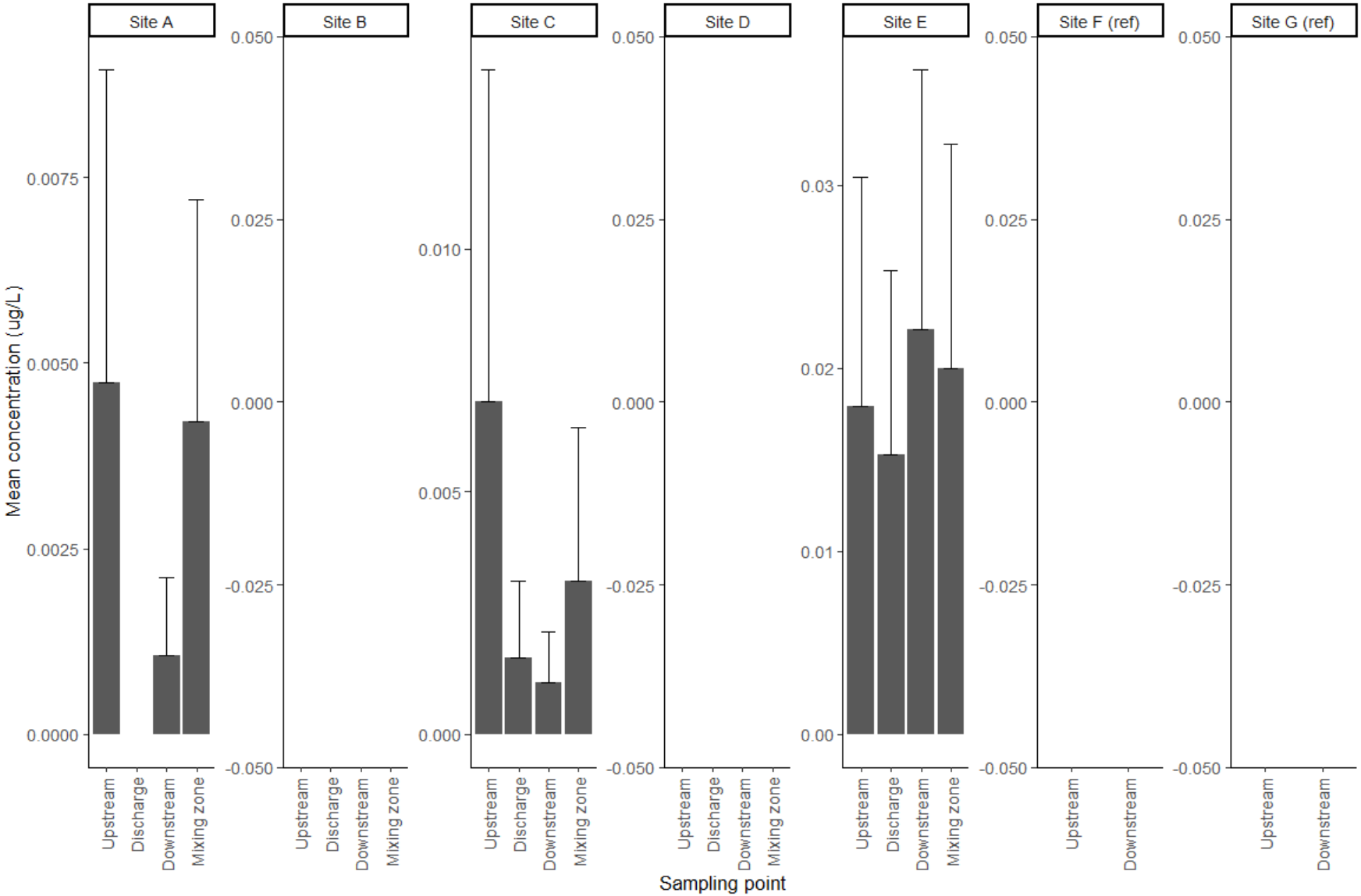

Figure 8. Mean concentrations (µg/L) of phthalates detected at each surface water sampling point separated by site (Sites A–F), including standard error bars.

4.4. Effects-based analysis of wastewater and surface waters (bioassays)

See Appendix A for a comprehensive report of the bioassay results, which are briefly summarised in this section.

4.4.1. BLT-Screen assay (general toxicity to bacteria)

The highest effect (i.e., highest toxicity to bacteria) was observed in the wastewater influent samples, with all influent samples showing responses above the ecological effects-based trigger values (ecoEBT). Wastewater treatment significantly reduced toxicity to bacteria (96-99% reduction), with all effluent samples showing response below the ecoEBT for all sites except for Site A. Furthermore, all surface water samples across all the effluent-receiving sites (Sites A–E) showed responses below the ecoEBT, with most samples showing no assay response (i.e., below the detection limit), indicating a low risk to the surface water environment. The BLT-Screen assay response was below the detection limit for all surface water samples at both reference sites (Sites F & G).

See Appendix A section 3.2 for detailed results from the BLT-Screen assay.

4.4.2. IPAM assay (photosynthesis inhibition due to herbicides)

The influent samples had the greatest response in the IPAM assay (i.e., greatest inhibition of photosynthesis) with all influent samples exceeding the ecoEBT across all sites for both the 2h-IPAM and 24h-IPAM. IPAM activity was reduced in the effluent samples compared to the influent samples for both the 2h-IPAM (75-98% reduction), and the 24h-IPAM (76-97% reduction). However, effluent at Site A for the 2h-IPAM assay, and effluent at Sites A and C for the 24h-IPAM assay exceeded the ecoEBT. That said, all surface water samples across all effluent-receiving sites (Sites A–E) were below the ecoEBT for both the 2h-IPAM and 24h-IPAM assays, indicating low risk to the surface water environment. Both the 2h-IPAM and 24h-IPAM assay responses were below the detection limit for all surface water samples at both reference sites (Sites F & G).

See Appendix A section 3.3 for detailed results from the IPAM assay.

4.4.3. ER-GeneBLAzer assay (estrogenic activity from EDCs)

Influent samples showed the highest estrogenic activity, with all sites showing responses above the ecoEBT. For effluent, estrogenic activity was reduced by 88-95% for Sites B–D, but only by 29% for Site A. However, regardless of the reduction, all effluent samples still showed responses above the ecoEBT. Surface water samples above the ecoEBT were also observed at all effluent-receiving sites except for Site B. Exceedances of the ecoEBT indicates a possible risk to the environment, although most surface water samples were less than 5 times the ecoEBT. However, the surface water sample at the discharge point from Site D was 25 times higher than the ecoEBT and could indicate a significant impact of estrogenic chemicals to the surface water environment at this site. All surface water samples at both reference sites (Sites F & G) were below the detection limit for this assay. Additionally, no anti-estrogenic activity was observed in any sample.

See Appendix A section 3.4 for detailed results from the ER-GeneBLAzer assay.

4.4.4. ARE-GeneBLAzer assay (general oxidative stress response)

Oxidative stress responses above the ecoEBT were detected in all influent and effluent samples, as well as 8 surface water samples across all effluent-receiving sites. The highest response was observed in the influent samples, with 79-96% reduction in effluent. Exceedances in surface water samples at Sites A, C and E were up to 2.5 times higher than the ecoEBT, indicating a potential risk to the environment at these sites. In contrast, all surface water samples from Sites B and D were below the ecoEBT, indicating low risk at these sites. All surface water samples from the reference sites (Sites F & G) were below the limit of detection for this assay.

See Appendix A section 3.5 for detailed results from the ARE-GeneBLAzer assay.

4.4.5. AhR-CAFLUX assay (presence of dioxin-like chemicals)

The highest AhR activity was observed in effluent at Site A, higher than the assay response seen in influent at this site. With the exception of Site A, AhR activity was reduced from influent to effluent samples at the other WWTP sites (Sites B–D) with between 50-91% efficiency, although a majority of these samples still exceeded the ecoEBT. Surface water samples also exceeded the ecoEBT at Site A by up to 1.4 times the ecoEBT, suggesting a potential risk at this site. In contrast, surface water samples were below the ecoEBT at the other effluent-receiving sites (Sites B–E), indicating low risk. All surface water samples from the reference sites (Sites F & G) were below the limit of detection for this assay.

See Appendix A section 3.6 for detailed results from the AhR-CAFLUX assay.

4.4.6. HiTMiN assay (genotoxicity)

All samples were below the assay limit of detection for genotoxicity. Influent samples across all sites, and effluent samples from Sites B and D were cytotoxic at higher concentrations. As there is a narrow window between genotoxicity and cytotoxicity, it is possible that genotoxic compounds were contributing to the observed cytotoxicity in these samples.

See Appendix A section 3.7 for detailed results from the HiTMiN assay.

Discussion

5.1. Prevalence and frequency of chemicals in wastewater samples

Of the 643 contaminants analysed, 143 were detected across influent, effluent and surface water samples, which included 4 artificial sweeteners, 11 EDCs, 36 pesticides, 18 PFAS, 6 phthalates, and 68 PPCPs. Compared to the previous 2021 EPA study (EPA Publication 2054; EPA, 2023), this study analysed a larger suite of contaminants (643 compared to 413 in 2021 study), but detected fewer contaminants (143 compared to 180 in 2021 study). It is important to note that the two studies targeted a slightly different group of chemicals (e.g. this study did not analyse phenols and disinfection by-products).

Of the 109 PPCPs analysed in effluent waters, 55 were detected, of which 28 were detected in 100% of effluent samples. This is consistent with the previous 2021 EPA study (EPA Publication 2054; EPA, 2023) with 100% detection rate in effluent observed for the antiepileptic medication lamotrigine and carbamazepine, the anxiety-relieving medication oxazepam and the medication used to treat insomnia temazepam in both studies. Lamotrigine and carbamazepine are consistently among the most prescribed antiepileptic drugs worldwide, in particular, due to increases in their uses to treat other afflictions such as migraines and mood disorders (reviewed in Cardoso-Vera et al., 2021; Liu et al., 2023). This increased consumption coupled with incomplete degradation via wastewater treatment processes has led to the occurrence of these PPCPs in the environment globally (Wilkinson et al., 2022). Similarly, benzodiazepines, a class of pharmaceuticals with anxiety-relieving properties, such as oxazepam and temazepam have been shown to be prescribed in large quantities globally and consequently, are increasingly reported as environmental contaminants worldwide (Fick et al., 2017; Kosjek et al., 2012; Wilkinson et al., 2022).

The most frequently detected artificial sweeteners in effluent were acesulfame K, cyclamate, saccharin and sucralose, all of which were detected in 100% of effluent samples. Additionally, sucralose was also detected at a 100% frequency in surface water sampling points downstream of effluent discharge (discharge point, and first and second downstream locations). In comparison to the previous 2021 EPA study (EPA, 2023), acesulfame K was also one of the most frequently (97%) detected compounds (classified as a PPCP in 2021 study) in effluent. It is important to note that the 2021 EPA study only

measured acesulfame K. In the current study, the highest effluent concentration of artificial sweeteners detected was for sucralose, which was consistently one to two orders of magnitude higher than the other detected artificial sweeteners. This result is also consistent with the poor estimated percent reduction (%R) of sucralose from influent to effluent observed in this study. Sucralose is a popular artificial sweetener that has been approved for use in over 80 countries, and is currently used in more than 4000 products as a non-caloric sweetener (Tollefsen et al., 2012). Although the increased intake of artificial sweeteners such as sucralose has been shown to play a role in the increasing rates of obesity and other health implications in humans (Singh S et al., 2024), studies on the impacts of these compounds as environmental contaminants are limited (Praveena et al., 2019).

The most frequently detected EDCs in effluent were bisphenol A and estrone, and the highest effluent concentration was for nonylphenol which was detected at an order of magnitude higher than the other EDCs. These results were consistent with the previous 2021 EPA study (EPA, 2023), where in effluent, nonylphenol, estrone and bisphenol A were the most frequently detected EDCs and nonylphenol had the highest concentration detected (0.54 µg/L). The uses and sources of EDCs can vary. For example, nonylphenol and bisphenol A are common industrial chemicals and originate from industrial sources and consumer products (Corrales et al., 2015; Soares et al., 2008), whereas estrogens such as estrone are either naturally produced by the body or taken as synthetic steroids, and are excreted in bodily wastes (Ying et al., 2002). Overall, EDCs are a group of emerging contaminants of concern because they are known to interfere with the endocrine system of animals, including humans (reviewed in Encarnação et al., 2019; Marlatt et al., 2022).

Several PFAS were detected in all effluent samples. These were PFPeA, PFHxA, PFHpA, PFOA, PFBS, PFOS, and PFHxS, with the highest concentrations detected for PFHxS and PFOS. In the previous 2021 EPA study (EPA, 2023), a similar trend in the frequency and types of PFAS in effluent was observed. However, trends in concentration levels were different between this study and the previous 2021 EPA study (EPA, 2023). Specifically, PFOS was only the seventh highest concentration detected in effluent for the previous study, while it had the second highest effluent concentration in this study. That said, other EPA studies which have analysed environmental samples from streams and rivers have found PFOS to consistently have the highest detected concentrations (Sardiña et al., 2019; EPA Publication 1879).

For pesticides, DEET, diuron and imidacloprid were detected in all effluent samples, with the highest concentrations detected for the repellent ingredient, DEET. This was contrary to the previous EPA 2021 study (EPA, 2023), where DEET was detected in only 29% of effluent samples and the triazine herbicide, simazine had the highest frequency of detection (82%) and maximum effluent concentration in that study. By comparison, in this study, simazine was only detected in 1 of 4 total effluent samples (25%) at a comparatively low concentration of 0.1 µg/L (just above the LOR). However, there were some similarities between studies. For example, the second and third most frequently detected pesticides for both this study and the 2021 EPA study (EPA, 2023) were diuron (79%) and imidacloprid (62%). Interestingly, pesticide contaminants screened at the Great Barrier Reef Catchment Area in Queensland, Australia found that both imidacloprid and diuron were also part of a group of five active ingredients that explained the majority of the pesticide mixture toxicity risk, indicating similarities in the use of pesticides between states in Australia (Neale et al., 2024; Spilsbury et al., 2020).

Unlike the other emerging contaminant groups analysed for in this study, no individual phthalate had a 100% detection rate in effluent samples. Instead, the most frequently detected phthalates were DEHP and DEP found in 50% of effluent samples, of which DEHP was found to have the highest effluent concentration. Phthalates were not analysed for in the previous 2021 EPA study (EPA, 2023), but were analysed in an environmental monitoring study during floods (Saaristo et al., 2024). These chemicals are widely used as plasticizers in the manufacture of plastics, and because they are only physically, and not chemically, bound to the produced matrix, they are easily dispersed and released into the environment

(Net et al., 2015). Some phthalates have also been shown to be endocrine disrupting chemicals, with potential developmental and reproductive impacts from exposure (Heudorf et al., 2007; Zhang et al., 2021).

5.2. Guideline values

Many emerging contaminants do not yet have water quality guidelines. Moreover, when guidelines exist for emerging contaminants, they are often set at concentrations much higher than those found in the environment. While many of these contaminants, such as PPCPs and EDCs, may not be directly toxic at the observed concentrations detected in the environment, they have been shown to induce sub-lethal effects, such as changes in metabolism, physiology and behaviour of non-target organisms (Ford et al., 2021; Saaristo et al., 2018).

Based on results of the targeted chemical analyses of this study, to provide a preliminary indication of risks to human health and the environment from wastewater discharges, we compared concentrations of detected compounds in effluent and/or surface water with guideline values from Australian sources, where available. The primary guidelines relevant to this study are ecological/ecosystem-based guidelines for water quality such as the ANZG toxicant default guideline values for aquatic ecosystem protection (ANZG, 2024), and the National Environmental Management Plan on PFAS (PFAS NEMP v3.0 draft; HEPA, 2022). Additionally, there are human health-based guidelines for recreational water such as the guidelines for managing risks in recreational water (NHMRC, 2008), and of lesser relevance to the sampling conducted in this study, for drinking water, such as the Australian Drinking Water Guidelines 6 (NHMRC and NRMMC, 2011).

5.2.1. PPCPs

There are currently no ecological or human health-based guidelines for PPCPs in Australia (ANZG, 2024; NHMRC and NRMMC, 2011).

5.2.2. Artificial sweeteners

There are currently no ecological or human health-based guidelines for artificial sweeteners as environmental contaminants in Australia (ANZG, 2024; NHMRC and NRMMC, 2011).

5.2.3. EDCs

Of the EDCs detected in this study, guideline values are only available for Bisphenol A for protection of ecosystems in freshwater and marine water (ANZG, 2024). Bisphenol A was only detected in effluent in this study, with none of the detected concentrations exceeding the most conservative 99% species protection exposure concentration of 0.78 µg/L for freshwater. Bisphenol A was not detected in any surface water samples, and therefore, there were no exceedances of guideline values for this compound in surface water.

5.2.4. PFAS

Ecological water quality guideline values for PFAS have been developed for PFOS and PFOA in Australia (ANZG, 2023; HEPA, 2022). The 99% protection guideline value is recommended for use by the Water Quality Guidelines (WQG) framework, as this is the adopted approach for chemicals that bioaccumulate and biomagnify in wildlife, such as PFAS (HEPA, 2022). For PFOS, the most stringent 99% species protection guideline value for freshwater systems (0.0091 µg/L; ANZG, 2023) was exceeded in effluent samples at Sites A and C, and in all surface water samples at Site A. These exceedances indicate potential risks of harm to wildlife due to exposure to PFOS in these receiving waterways. For PFOA, the 99% species protection guideline value for both freshwater and marine systems (19 µg/L) was not

exceeded in any effluent or surface water samples across all sites, including reference sites (Sites A–G) (HEPA, 2022).

For human health-based guideline values for PFAS, recreational water quality guideline values have been developed for the sum of PFOS and PFHxS (2 µg/L), and for PFOA (10 µg/L) (HEPA, 2022). For PFOA, like the ecological guideline values, there were no exceedances for recreational water quality in effluent or surface water. For the sum of PFOS and PFHxS, no exceedances for recreational water quality in effluent or surface water were observed.

5.2.5. Pesticides

Ecological and human health-based guidelines exist for a range of pesticides (ANZG, 2024; NHMRC, 2008). For freshwater and marine water ecological guideline values (ANZG, 2024), exceedances were observed for two pesticides in effluent and surface water, as follows:

1. Metolachlor, an aniline-derived herbicide typically used to control grass and broadleaf weeds, was detected in one surface water sample (upstream at Site E) just above the LOR at 0.01 µg/L, which exceeds the 99% species protection guideline value of 0.0084 µg/L for freshwater, but not the 95% species protection guideline value of 0.46µg/L for slightly to moderately disturbed freshwater systems.
2. Metsulfuron-methyl, a sulfonyl-urea herbicide also used to control grasses and broadleaf weeds, exceeded the 99% freshwater guideline value of 0.0037 µg/L in one effluent sample (0.01 µg/L, Site A), and in all surface water samples at Sites A and E and the discharge point at Site C (max. of 0.11 µg/L detected). The 95% freshwater guideline value of 0.018 µg/L was also exceeded in two surface water locations at Site A (upstream and discharge), at the discharge point at Site C, and at all surface water locations at Site E. Exceedances of guideline values at upstream surface water sampling locations for this pesticide indicate that effluent discharge may not be the primary contributor to the receiving environment but could be entering the environment via other surrounding diffuse sources.

For human-health based guideline values for recreational water quality (NHMRC, 2008) and drinking water quality (NHMRC and NRMMC, 2011), there were no exceedances for any of the detected pesticides in effluent and surface water.

5.2.6. Phthalates

Ecological and human-health based guidelines exist for several phthalates, including DEHP, DBP, DEP and DMP (ANZG, 2024; NHMRC and NRMMC, 2011). For DEHP, the freshwater guideline value (1 µg/L with unknown reliability) was exceeded only in effluent at Site C (7.6 µg/L), but not in any surface water samples. None of the other detected phthalates exceeded ecological guideline values for freshwater or marine water. For human health-based guideline values, only DEHP has a drinking water guideline value of 10 µg/L (NHMRC and NRMMC, 2011) which was not exceeded in any effluent or surface water samples.

5.3. Comparisons of trends between targeted chemical analyses and effects-based analyses of surface waters, and the utility of bioassays in risk assessment

Overall, the bioassay results (Appendix A) show that the water quality in surface water upstream and downstream of the WWTPs was acceptable for most assay endpoints, with most samples below the ecological effects-based threshold (ecoEBT) guideline value. However, some surface water samples exceeded the recommended ecoEBT guideline values for specific assays, which may indicate potential risks to the receiving environment and warrant further investigation.

When comparing bioassay results to the targeted chemical analyses for surface water, comparable trends can be observed. For example, high overall contaminant concentrations were detected at Sites C

and E, and a resulting exceedance of the bioassay ecoEBTs for oxidative stress (ARE-GeneBLAzer bioassay) was also reported at these sites, suggesting potential ecological impacts from contaminant loads. Specifically, for the bioassay results that indicate general toxicity from overall contaminant load (the BLT-Screen and ARE-GeneBLAzer assays), only three surface water samples across all sites were observed above the limit of detection for toxicity to bacteria in the BLT-Screen assay, but none of these exceeded the ecoEBT guideline value, indicating low risk to the receiving environment for acute toxicity overall. However, with the ARE-GeneBLAzer assay for general oxidative stress, exceedances of the ecoEBT guideline value (up to 2.5 times higher) were observed for all surface water samples at Site E, for the upstream sample at Site A, and for 3 of the 4 surface water samples at Site C, which suggests a potential ecological risk to the receiving environment and would warrant further investigation in a risk assessment setting. In contrast, none of the surface water samples at Sites B and D, or the two reference sites (Sites F and G) exceeded the ecoEBT, indicating a low risk there. Oxidative stress responses are generally a sign of a body's immune response to a foreign contaminant (Valavanidis et al., 2006), and therefore would be more sensitive than acute toxicity endpoints (such as in the BLT-Screen) and can be induced by exposure to many low potency chemicals, including pesticides and PPCPs.

Additionally, bioassay responses can be indicative of contaminant stress from specific chemical groups that have a specific mode of action, and may therefore, be useful in determining the types of contaminants that pose ecological or health risks. For example, the IPAM assay targets herbicidal, and more broadly, pesticidal modes of action, and can be representative of the potential harm that herbicides and other pesticides can have on the surface water environment. For the IPAM assay results, although many surface water samples, particularly at Sites A, C, and E, were above the limit of detection for the bioassay response, none of these exceeded the ecoEBT guideline value. The lack of exceedances of the ecoEBT for this bioassay in surface water at these sites suggests that herbicidal and pesticidal activity due to the presence of contaminants may be of an overall low risk to the receiving environment. Again, the pesticide detection trends in surface water observed with targeted chemical analyses and an induced response of photosynthesis inhibition in the bioassay were comparable. Specifically, pesticides were detected only at Sites A, C and E, and similarly, an IPAM assay response in surface water samples was observed only at these sites.

In our study, the ER-GeneBLAzer bioassay that tests for estrogenic and anti-estrogenic activity had the most exceedances of the trigger value. Specifically, all surface water samples collected at or downstream of the effluent discharge exceeded ecoEBT guideline values for this assay at Sites A and E. Also, several downstream surface water samples at Sites C and D exceeded the ecoEBT, at up to 25 times, indicating a higher risk. Generally, exceedances of over 10 times the ecoEBT warrants further action to identify causes, if not explained by targeted chemical analyses. It is also important to note that across all upstream sampling locations, only the upstream surface water sample at Site A exceeded the ecoEBT guideline value for this assay, suggesting that effluent discharge may be contributing to endocrine disrupting impacts at most of the effluent-receiving sites. Interestingly, although surface water samples at all sites except for Site B was found to induce estrogenic responses above the ecoEBT, targeted chemical analyses showed that contaminant loads of EDCs was only high in surface water at Site C. This may indicate that the estrogenic bioassay responses seen across the effluent-receiving sites may be due in part to contamination by unknown chemicals not currently targeted in the chemical analyses, or by other chemical groups captured in the chemical analyses that have been shown to also induce estrogenicity (e.g. phthalates). Therefore, to improve assessment of risk, further investigation may be needed to uncover the mixture effects.

Overall, the bioassay results also show that wastewater treatment processes can remove the majority of contaminants from influent streams, with most sites achieving over 80% removal of bacterial toxicity, photosynthesis inhibition, estrogenic activity and oxidative stress. However, removal efficiencies for activity in the bioassays differed across sites and between contaminant groups, with particularly poor

removal of estrogenic activity and dioxin-like activity at Site A. This supports the estimated percent reduction (%R) of emerging contaminants observed from the targeted chemical analyses, which also showed that the majority of chemical groups had, on average, over 50% removal during wastewater treatment, but this was compound-specific with some chemicals in each contaminant group showing an increase in concentration following treatment.

Further recommendations, discussion and conclusions on the utility of bioassays in assessing water quality can be found in Appendix A and references therein.

Conclusions

This study showed that a wide range of PPCPs, some EDCs, pesticides, PFAS, phthalates and artificial sweeteners are present in wastewater effluent. This is consistent with the findings of the previous 2021 EPA study (EPA Publication 2054; EPA, 2023). For most of the emerging contaminants detected, concentrations were lower in effluent than in influent. However, this was not true for PFAS which had a higher mean concentration in effluent than influent. Reduction of contaminants through wastewater treatment processes was compound-specific, and although removal efficiencies were high for many chemicals, some contaminants (e.g. some pharmaceuticals) passed through treatment trains relatively untreated.

For trends of contaminant load in surface waters, a significant increase was observed from upstream of discharge to downstream sites away from discharge for PPCPs, artificial sweeteners and EDCs. This indicates that, in general, WWTP discharge seems to be the primary source of these contaminants, although site-specific trends can vary. On the contrary, pesticides and PFAS were detected in both upstream and downstream sites, indicating multiple input sources. Lastly, no clear trends were observed for phthalates, with mean concentrations of phthalates found to be highest upstream of the effluent discharge, suggesting that effluent discharge may not be the main source of phthalates into the environment.

The identification of risks to surface water environments from emerging contaminants in effluent discharge was one of the main knowledge gaps identified in the 2021 EPA study (EPA Publication 2054; EPA, 2023). To address this, one of the primary aims of this study was to develop an understanding of the use of bioassays in helping to determine and quantify risks of emerging contaminants to the environment. In that regard, the bioassay results showed that wastewater treatment can remove the majority of active chemicals. However, both the bioassay results and the targeted chemical analyses highlighted that removal was compound specific. The bioassays further demonstrated that there were lower removal efficiencies for chemicals that induce estrogenic activity and dioxin-like activity. With the data from the bioassays, we can now identify potential ecological risks of harm to the receiving surface water environment from overall contaminant load at specific sites, and from specific contaminant groups (such as endocrine disrupting chemicals and dioxin-like chemicals).

Overall, the results of this study enhanced the state of knowledge for the Victorian water sector by providing a better understanding and baseline data on the prevalence of emerging contaminants in wastewater and wastewater discharges to the surface water environment in Victoria. This study provides valuable information for the water sector regarding development of Risk Management and Monitoring Programs (RMMPs) and meeting their GED requirements. This study will feed into future licence reviews by providing valuable insights into removal efficiencies. Altogether, this study demonstrates that bioassays offer a holistic approach to assessing risks of emerging contaminants to the environment.

How to use bioassays and bioassay data

Effects-based methods (EBMs) such as the bioassays used here, focus on observing actual biological responses. Therefore, the combination of targeted chemical analyses and EBMs enable assessment of synergic or cumulative effects from the mixtures of emerging contaminants that may be present in water. For example, some chemicals may be present at low levels yet still be biologically active. Therefore, a battery of multiple bioassays that cover response to general contamination, as well as response to specific modes of actions (e.g., estrogenic activity), should be used to effectively manage water quality and ecological health.

Using a combination of targeted chemical analysis and biological data from bioassays can support the water industry and water professionals in several ways, including:

- As weight-of-evidence approach to characterise water quality in monitoring programs;
- Used as a screening tool to focus resources on where biological effects are observed, or to identify 'hotspots' or sources of cumulative impacts;
- To validate control measures and prioritise risks to be managed in Risk Management and Monitoring Programs (RMMPs) plans.
- Supplement targeted chemical monitoring, especially where guideline exceedances are not observed but adverse effects to the non-target organisms may be present;
- To identify and assess chemical hazards in environmental flows, discharge loads and risks to sensitive receptors in site-specific risk assessments;
- To test if implemented control measures (e.g. improved treatment or source control) are effective and fit for purpose.

The results of EBMs are now routinely expressed as standardised units, and can typically be compared between different bioassays, different sites, and different studies, to identify trends in water quality.

Detecting a bioassay response does not always mean there is an unacceptable risk. Effects-based trigger values (EBTs) have been developed to help differentiate between acceptable and unacceptable bioassay responses (both from an ecological or human health perspective), similar to individual chemical guideline values for water quality. Following the observation of an exceedance of the EBT for a specific bioassay, an operational response can then be taken in steps to further address the issue. Briefly, these steps may involve bioassay quality control checking and retesting of samples to validate results, targeted analysis of known potent chemicals where bioassays have exceeded EBTs for a specific mode of action, determining if cytotoxicity of samples exceeds an acceptable level for bioassays in which many low-potency chemicals contribute to an exceeded EBT response, and potentially the optimising of treatment processes where risks may be deemed unacceptable.

For further general information on the application of effects-based methods in water quality monitoring, see (Neale et al., 2023a). For the derivation and use of EBTs, see (Neale et al., 2023b) and references therein. On the integration of EBMs into water safety plans, see (Neale et al., 2022).

Limitations

- The study timeframe was limited, with the sampling conducted between the 17th of April 2024 to the 12th of June 2024.
- The results represent a snapshot in time, with a single sample taken for each sampling point at each site.

- Variations in the prevalence and concentrations of specific chemicals may be apparent due to:
    - Seasonal patterns of chemical use
    - Temperature influences on wastewater treatment efficacy and environmental fate
    - Diurnal patterns for specific chemicals
    - Increases in use of specific chemicals based on specific occurrences of events
- The bioassay used to determine the genotoxicity of mixtures (HiTMiN assay) was still relatively new and further method development would be needed to be able to differentiate between toxicity due to genotoxicity or cytotoxicity, as genotoxic compounds can contribute to cytotoxicity.

Future directions and priorities

- Development of guidance on bioassays as a weight-of-evidence approach for the assessment of risks of emerging contaminants discharged into receiving environments, to better enable the management of risks and responsibilities under the general environmental duty (GED).
- Further investigation into use of bioassays across different treatment trains to build up baseline information for discharge of emerging contaminants into surface water environments.
- Update of VicWater 2019 risk assessment for treatment trains across Victoria to cover more sites. Currently there are several sites without an allocated treatment train rating and therefore uncertain risk profile.
- Drinking water catchments require further investigation regarding the presence of emerging contaminants.
- Wider application (e.g. agricultural settings) of the use of bioassays to assess risk of recycled water reuse are warranted.
- There is limited understanding of the ecological and population level impacts of emerging contaminants in Victorian waterways.

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Appendix A – Bioassay report

Griffith UNIVERSITY
Queensland, Australia

FINAL REPORT

Effect-based analysis of wastewater and surface waters from Victoria

Frederic Leusch, Peta Neale and Elissa O'Malley

Project 59566 Final Report – 19 December 2024

Effect-based analysis of wastewater and surface waters from Victoria

Project 59566

FINAL REPORT – 19 December 2024

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Contents

Executive summary

The aquatic environment can contain a complex mixture of many chemicals, often present at low concentrations. Targeted chemical analysis, which is typically used for chemical water quality monitoring, can only detect a small fraction of the total chemical burden. Consequently, effect-based methods (EBM) can be applied complementary to chemical analysis to detect the effect of all active chemicals in a sample. In this study, a battery of *in vitro* bioassays indicative of bacterial toxicity, photosynthesis inhibition, estrogenic activity, oxidative stress response, activation of the aryl hydrocarbon receptor (AhR) and genotoxicity were applied to water samples collected from four wastewater treatment plants (WWTPs) as well as surface water upstream and downstream of the WWTPs. Surface water from two control sites was also tested. The observed effect was compared with previously reported activity from the literature and ecological effect-based trigger values (ecoEBT).

All wastewater influent and effluent samples had a response in assays indicative of bacterial toxicity, photosynthesis inhibition, estrogenic activity, oxidative stress response and activation of AhR. In contrast, none of the samples were genotoxic in the HiTMiN assay. Three of the WWTPs, Site B, Site C and Site D , were able to remove over 80% of bacterial toxicity, photosynthesis inhibition, estrogenic activity and oxidative stress response. Lower removal efficiency was consistently observed for the Site A WWTP, with particularly poor removal of estrogenic activity and AhR activity.

Few surface water samples induced bacterial toxicity, while the effect in most surface water samples was close to the limit of detection in the photosynthesis inhibition assay. Estrogenic activity, AhR activity and induction of the oxidative stress response were more commonly observed in surface water, with the effect in some of the samples exceeding the ecoEBT. In contrast, the control site samples did not induce a response in any of the bioassays.

Overall, the data show that the water quality upstream and downstream of the WWTPs was good (below ecoEBT) for all endpoints at Site B and the control sites (Sites F and G). Surface water quality for the Site D site was good for all endpoints, except estrogenic activity. Higher estrogenic activity was also observed in the surface water from Site A. The observed effects similar to previously reported activity for surface water globally. None of the surface water samples exceeded the ecoEBT for the bacterial toxicity or photosynthesis inhibition assays, indicating a low risk. Some of the surface water samples slightly exceeded the ecoEBT for assays indicative of oxidative stress response (53% of samples above assay detection limit) and activation of AhR (27% of samples above assay detection limit), but not by more than 1.4 to 2.5 times. In contrast, estrogenic activity posed a higher risk, with one surface water sample exceeding the ecoEBT by over 25 times. Further, the effluent from Site A WWTP also showed high estrogenic activity and indicated a possible risk to the environment, particularly if not adequately diluted in the receiving environment.

1 Introduction

The aquatic environment can contain numerous chemical contaminants, including pesticides, pharmaceuticals and industrial compounds, with wastewater discharges considered a major contributor to chemical pollution in surface waters (Neale et al., 2017). Targeted chemical analysis is typically used to monitor chemical water quality, but the countless number of chemicals present, including chemical transformation products, means that targeted chemical analysis will only detect a small fraction of the total chemical burden. Further, chemical analysis cannot account for the mixture effects that occur between different chemicals. As a result, bioanalytical tools, which are also known as *in vitro* bioassays or effect-based methods (EBMs), can be applied complementary to targeted chemical analysis and are recommended for water quality monitoring (Brack et al., 2019). This is because EBMs can detect the mixture effects of known and unknown chemicals and are risk scaled, with potent chemicals having a greater effect. EBMs are high-throughput cell-based bioassays and can detect the effect of all active chemicals in a sample.

EBMs indicative of different stages of cellular toxicity pathways, including induction of xenobiotic metabolism (e.g. activation of the aryl hydrocarbon receptor (AhR)), receptor-mediated effects (e.g. activation of the estrogen receptor, photosynthesis inhibition), reactive toxicity (e.g. genotoxicity), adaptive stress responses (e.g. oxidative stress response) and apical effects (e.g. non-specific toxicity to bacteria) have been widely used for water quality monitoring, as reviewed in Escher et al. (2021) and Neale et al. (2023a).

Similar to guideline values for known chemicals, effect-based trigger values (EBTs) are used to determine whether the chemical water quality is acceptable or not. The observed effect in a bioassay can be compared to a bioassay specific EBT, with EBTs derived for both human health (e.g., drinking water) and ecosystem health (e.g., surface water) (Brand et al., 2013; Escher et al., 2018; Escher et al., 2015; van der Oost et al., 2017). Guidance on what to do if the effect in a sample exceeds an EBT is provided in Neale et al. (2023b).

This report presents the bioanalysis results to date for four wastewater treatment plants (WWTPs), surface water upstream and downstream of the WWTPs and two with control sites. The results are benchmarked against the current literature and compared with available ecological EBTs.

2 Methodology

2.1 Sampling Locations

Influent and effluent samples were collected from four WWTPs in Victoria, as well as from creeks and streams upstream and downstream of the WWTPs. In addition, two control ('pristine') sites were sampled. The sampling locations are described in Table 1.

Table 1. Sample location, description and sampling dates.

Site ID	Location	Sample Date	Sample ID	Sample Description
1	Site A WWTP	17/04/2024	1_1	Site A WWTP - Influent
			1_2	Site A WWTP - Effluent
			1_3	Site A WWTP - Up
			1_4	Site A WWTP - Down
			1_5	Site A WWTP - Discharge
			1_6	Site A WWTP - Mix
			1_7	Ultrapure water control
4	Site B WWTP	30/04/2024	4_1	Site B WWTP - Influent
			4_2	Site B WWTP - Effluent
			4_3	Site B WWTP - Up
			4_4	Site B WWTP - Down
			4_5	Site B WWTP - Discharge
			4_6	Site B WWTP – Mix
2	Site C WWTP	22 – 23/04/2024	2_1	Site C WWTP - Influent
			2_2	Site C WWTP - Effluent
			2_3	Site C WWTP - Up
			2_4	Site C WWTP - Down
			2_5	Site C WWTP - Discharge
			2_6	Site C WWTP - Mix
			2_7	Ultrapure water control
3	Site D WWTP	29 – 30/04/2024	3_1	Site D WWTP - Influent
			3_2	Site D WWTP - Effluent
			3_3	Site D WWTP - Up
			3_4	Site D WWTP - Down
			3_5	Site D WWTP - Discharge
			3_6	Site D WWTP – Mix
6	Site E	12/06/2024	6_1	Site E - UP
			6_2	Site E - Down
			6_3	Site E - Discharge
			6_4	Site E - Mix
5	Site F and Site G	01/05/2024	5_1	Site F - Up
			5_2	Site F - Down
			5_3	Site G - Up
			5_4	Site G - Down

2.2 Sample Processing

Sample bottles were pre-cleaned by rinsing twice with methanol and left to evaporate overnight. Prior to sample collection, the sample bottles were rinsed with water from the collection site. 24h-Composite samples were collected for the influent and effluent samples, with grab samples collected for all other samples. Samples were collected in 1 L amber glass bottles, kept cold on ice and immediately shipped for processing within 48h of collection to the Griffith University laboratory.

Upon arrival, samples were brought to room temperature then immediately filtered using a vacuum filtration apparatus. Samples were passed through a glass fibre pre-filter (Merck Millipore), followed by a 0.7 µm glass microfibre filter (Whatman), a 0.45 µm polyethersulfone (PES) membrane filter and a 0.2 µm PES membrane filter (Sterlitech). If required, samples were refrigerated overnight prior to extraction.

Samples were then passed through pre-conditioned Oasis HLB Solid Phase Extraction (SPE) cartridges (Waters, 186000115) using an automated SPE workstation (Promochrom SPE-03, Promochrom Technologies). SPE cartridges were pre-conditioned with 2× 5 mL acetone:hexane, 2× 5 mL methanol, and equilibrated with ultrapure water (Milli-Q EQ 7000, 0.22 µm, Millipak). Water samples were drawn through the SPE cartridge at a maximum rate of 10 mL/min. Cartridges were then dried and eluted with 5 mL methanol and 5 mL acetone:hexane. The eluates were blown down to dryness, reconstituted in 500 µL methanol, which gave a relative enrichment factor 2000×, and transferred to a 2 mL HPLC amber glass vial (Agilent Technologies 5182-0716). These concentrated sample extracts were kept at -20°C while awaiting bioassay analysis.

2.3 Bioassay Analysis

A battery of six bioassays was applied to screen extracts for chemical-associated toxicity: BLT-Screen for bacterial toxicity due to a range of organic micropollutants, IPAM for photosynthesis inhibition due to herbicides, ER-GeneBLAzer for estrogenic endocrine disrupting compounds, HIiTMiN assay for genotoxic and cytotoxic chemicals, ARE-GeneBLAzer for oxidative stress, and AhR-CAFLUX for dioxin-like compounds and pesticides.

The BLT-Screen assay, a measure of toxicity to bacteria, was conducted as previously described (van de Merwe and Leusch, 2015). In brief, naturally bioluminescent *Photobacterium leiognathi* bacteria (ATCC 33469) were seeded in 96-well plates and exposed to serial dilutions of the sample for 30 min. After exposure, luminescence from each well was read in a plate reader (BMG FLUOstar Omega). Inhibition of luminescence is a measure of bacterial toxicity. As bacteria are critical to many biogeochemical cycles, toxicity to bacteria can have far-reaching ecological consequences. A wide range of organic micropollutants can adversely affect bacteria, and this assay does not specifically

detect antibacterial compounds - although of course they are particularly potent here. The BLT-Screen is a useful measure of overall water quality and allows benchmarking of the water samples to previously established water quality bands.

The IPAM assay quantifies inhibition of photosynthesis and toxicity to algae, and was carried out as described in Escher et al. (2008). In brief, *Raphidocelis subcapitata* freshwater algae were added to each well of a 96-well microplate at an optical density of 0.1 absorbance unit (AU) and exposed to serial dilutions of the sample extracts. A Maxi-IPAM camera (WALZ, Germany) was then used to measure photosynthetic capacity at 0, 2 and 24h, which was then used to calculate photosynthesis inhibition due to exposure to the sample extracts. Photosynthesis is the primary energy source in almost all biomes on Earth, and disruption of photosynthesis can thus have wide-ranging ecological consequences. Photosynthesis inhibition in water samples is usually well correlated with PSII herbicides such as diuron, simazine, and atrazine.

The GeneBLAzer bioassays (ER and ARE), which measure estrogen (ER) agonism and antagonism and induction of the anti-oxidant response element (ARE), respectively, were carried as previously described (Escher et al., 2014). In brief, GeneBLAzer ERα-UAS-*bla* GripTite and CellSensor ARE-*bla* Hep G2 cells (ThermoFischer Scientific, K1688 and K1633, respectively) were seeded in 384-well plates, immediately exposed to serial dilutions of the sample extracts, and incubated for 16h. After incubation, fluorescence in each well was read on a microplate reader (Tecan Spark) at λex = 410 nm and λem = 460 (blue) and 520 nm (green). The blue/green ratio was then used to quantify receptor-mediated reporter gene induction due to exposure to the sample extracts. Interference with ER indicates a potential to interfere with the normal function of the endocrine system in animals (including humans) caused by estrogenic endocrine disrupting compounds (EDCs), which can have impacts on reproduction, development, behaviour, metabolism, immune response and other key biological functions that require cellular communication. Estrogenicity is commonly detected in wastewater, and has been linked to intersex in exposed aquatic animals such as fish and gastropods. The ARE-GeneBLAzer assay is a measure of oxidative stress, which can be caused by a variety of chemicals. Similar to the BLT-Screen, a response in the ARE-GeneBLAzer assay can be benchmarked to previously established water quality bands to determine overall (chemical) water quality and determine the suitability of the water for various uses.

The AhR-CAFLUX, which measures induction of the aryl hydrocarbon receptor via a green fluorescent protein reporter (Nagy et al., 2002), was carried out as previous described using the mouse H1G1.1c3 cell line. Briefly, cells were seeded in 96-well microplates and incubated for 24h, and subsequently exposed to samples for 24h. Fluorescence in each well was read on a microplate reader (Tecan Spark) at 0 and 24h. Induction of the AhR is indicative of the presence of dioxin-like chemicals, which are highly toxic and persistent organic pollutants produced by natural and anthropogenic combustion that can

cause a wide range of negative health effects including cancer, immune and hormone system dysfunction, and broad reproductive and developmental issues.

The high-throughput micronucleus (HiTMiN) assay was conducted as described in Johnson et al. (2022), with minor modifications: RTgill-W1 cells (ATCC CRL-2523) were seeded at an increased density of 3000 cells/well, and 16 fields per well were obtained (at 20× magnification) leading to an increase in analysed cells. Images were captured using an EVOS M7000 Imaging System and analysed using Celleste 6 Image Analysis Software (ThermoFisher Scientific). In brief, the assay relies on high-throughput cell imaging to analyse micronucleus formation in cells exposed to serial dilutions of the test sample. Micronucleus formation is a well-recognised measure of DNA damage (genotoxicity), which may result in a somatic mutation and lead to malignant transformation (cancer).

2.4 Quality Assurance Quality Control (QAQC) and Data Analysis

All bioassays were run on at least two independent occasions. Quality Assurance Quality Control (QAQC) protocols involve the inclusion of a full concentration-effect curve with a reference compound, a positive control and a negative control in each run, and comparison of the concentration-effect curve of the reference compound with Shewart control data for each assay. Runs where the EC_{50} of the reference compound was more than 2 standard deviations away from the historical average were rejected and the assay re-run.

For each sample, the relative enrichment factor (REF), calculated as the product of the SPE enrichment factor and the assay dilution factor, was plotted against the response in each bioassay to produce a concentration-effect curve for each sample. The activity threshold, *i.e.* EC_{10} (ER, IPAM and AhR assays), EC_{20} (BLT-Screen), IC_{20} (ER-anta) or $EC_{IR1.5}$ (ARE and HiTMiN assays), was computed using non-linear regression of the concentration-effect curve and expressed in REF. For the BLT-Screen, bioactivity was expressed as a toxic unit (TU), calculated as $1/EC_{20}$(REF), while the bioassay response in HiTMiN was expressed as a genotoxic unit (GTU), calculated as $1/EC_{IR1.5}$(REF). For all other assays, the bioactivity was expressed as a bioanalytical equivalent concentration (BEQ), calculated as:

$$\mathrm{BEQ} = \frac{\text{activity threshold (of the reference compound)}}{\text{activity threshold (of the sample,in REF)}}$$

When there was a large discrepancy between assay runs for a sample, the sample was run a third time, with the average of the two closest runs used.

The reference compound, activity threshold and reporting unit for all assays used in this study are presented in Table 2.

As one of the ultrapure water controls had a low effect in the AhR-CALUX assay, blank corrected BEQ_{bio} values were presented. These were calculated by subtracting the average BEQ_{bio} of the two ultrapure water controls from the BEQ_{bio} of the sample. Standard deviation for the SPE blank corrected sample was calculated using error propagation. Any sample where the blank effect was more than 50% of the sample effect was excluded and reported as below detection limit. This is consistent with the previous approach in the literature (Rauert et al., 2024).

Table 2. Reference compound, activity threshold and reporting unit for the different bioassays used in this study.

Bioassay	Reference compound	Detection threshold	Results expressed as
BLT-Screen	Pentacholorophenol	0.06 TU_{20}	Toxic Unit (TU) at EC_{20}
IPAM (2h)	Diuron	0.01 µg/L	µg Diuron Equivalent (DEQ) / L
IPAM (24h)	Diuron	0.01 µg/L	µg Diuron Equivalent (DEQ) / L
ER-GeneBLAzer	17β-Estradiol	0.1 ng/L	ng Estradiol Equivalent (EEQ) / L
ER-GeneBLAzer (anta)	4-Hydroxytamoxifen (TMX)	193 ng/L	ng TMX Equivalent (TMXEQ) / L
ARE-GeneBLAzer	Dichlorvos (DDVP)	193 µg/L	µg Dichlorvos Equivalent (DDVPEQ) / L
AhR-CAFLUX*	TCDD Diuron	18 pg/L 1 µg/L	pg TCDD Equivalent (TCDDEQ) / L µg Diuron Equivalent (DEQ) / L
HiTMiN	Sodium chromate	0.05 GTU	Genotoxic Unit (GTU)

*Results for AhR-CAFLUX are typically expressed as TCDDEQ. Due to concerns about working with highly toxic TCDD, the herbicide diuron, which is active in AhR-CAFLUX and commonly detected in water samples, is now used as the assay reference compound. Therefore, the results are expressed as both TCDDEQ and DEQ.

2.5 Removal efficacy

The ability of the studied WWTPs to remove biological activity was assessed by comparing the BEQ, TU or GTU of the influent and the BEQ of the effluent using the following equation:

$$\text{Effect removal}\ (\%) = \frac{\text{BEQ}_{\text{influent}} - \text{BEQ}_{\text{effluent}}}{\text{BEQ}_{\text{influent}}} \text{ or } \frac{\text{(G)TU}_{\text{influent}} - \text{(G)TU}_{\text{effluent}}}{\text{(G)TU}_{\text{influent}}}$$

2.6 Comparison with Literature and Effect-Based Trigger Values

To allow interpretation of the results, the data are compared to ranges in the literature (summarised in Chapter 10 in Escher et al., 2021) for raw wastewater (WW), treated wastewater (TWW) and surface water (SW), as well as reported removal efficacy, where available. Data are also compared to available effect-based trigger (EBT) values, which provide a recommended value that is protective of ecosystem health (ecoEBT). ecoEBT values are sourced from Chapter 13 in Escher et al. (2021) and other sources

(cited in the text), and when unavailable were calculated using the methodology outlined in Neale et al. (2023b).

3 Results and Discussion

3.1 Overall trends

Overall, the data show that the water quality in surface water upstream and downstream of the WWTPs was mostly good for the majority of studied endpoints, with the scale of the bioassay responses similar to what is typically observed in wastewater or surface water globally. All assays had a response in at least one sample, except the HiTMiN assay for genotoxicity, where samples were either cytotoxic or below the assay detection limit. The control sites, Site F and Site G, did not induce a response in any of the bioassays.

While all wastewater samples had a response in the bacterial toxicity assay, only three surface water samples had a response above the limit of detection. All wastewater and 10 of the 24 surface water samples had a response in the photosynthesis inhibition assay, though the effect in the surface water samples was often close to the assay limit of detection. All surface water samples above the assay detection limit in the bacterial toxicity and photosynthesis inhibition assays were below the ecoEBT.

Unsurprisingly, estrogenic activity was detected in all wastewater samples. Estrogenic activity was also detected in 12 of the surface water samples, including all surface water samples from Site A. Many of the surface water samples exceeded the ecoEBT. There was no anti-estrogenic activity in any of the samples, as is typically reported for water samples.

All wastewater influent and effluent samples and 15 of the surface water samples had a response in the oxidative stress response assay. Many low potency chemicals, including pesticides, pharmaceuticals and industrial chemicals, can induce the oxidative stress response. Some of the surface water samples from Sites A, C and E exceeded the ecoEBT.

All wastewater influent and effluent samples as well as 11 of the surface water samples had a response in the activation of AhR assay (after blank correction), with most surface water samples from Site A slightly exceeding the ecoEBT.

Removal efficacy was calculated, with removal by the WWTPs comparable to previously reported removal efficacies in the literature. Over 80% removal of bacterial toxicity, photosynthesis inhibition, estrogenic activity and oxidative stress response was observed for the Sites B, C and D WWTPs. Site A WWTP typically had the lowest removal efficacy, with poor removal of estrogenic activity (29%) and AhR activity (-84%).

In terms of ecological health risk, none of the surface water samples exceeded the ecoEBT for bacterial toxicity or photosynthesis inhibition, indicating a low risk. Further, assuming a 10 times dilution into surface water, none of the WWTP effluent samples would exceed the ecoEBT in these assays. While

some surface waters slightly exceeded the ecoEBT for activation of AhR and oxidative stress response, the observed effect was only up to 1.4 and 2.5 times higher than the ecoEBT, respectively, which suggests a possible risk. In contrast, estrogenic activity posed a higher risk, with the discharge (3_5) sample from Site D over 25 times higher than the ecoEBT. Further, the effluent from Site A WWTP would need to be diluted over 100 times in the receiving environment to be below the ecoEBT.

3.2 BLT-Screen

Results: The highest effect was observed in the wastewater influent samples, with TU_{20} ranging from 6.6 to 25.4 (Figure 1 and Table 3). Wastewater treatment at all four plants significantly reduced bacterial toxicity, with TU_{20} ranging from 0.12 to 0.32. This equates to a 96 to 99% removal of bacterial toxicity after treatment. The treated effluent was below the ecoEBT for three of the WWTPs, with the effluent from Site A slightly above the ecoEBT. However, this would be below the ecoEBT after 10 times dilution into surface water. All surface water samples were below the ecoEBT, indicating a low risk. The surface water and treated wastewater samples were within a similar range to previously detected, though the effect in wastewater influent was higher than previously reported (note that BLT-Screen not previously applied to wastewater influent, though the Microtox assay has).

Effect based trigger values: The BLT-Screen ecoEBT is 0.2 TU_{20}. This is based on a TU_{50} of 0.05 or an EC_{50} of REF_{20} (van der Oost et al., 2017). The EC_{50} was converted to an EC_{20} of 5 assuming a slope of 1, with the EC_{20} converted to TU_{20}.

Typical range: The typical range in different water types for BLT-Screen reported in Escher et al. (2021) include:

- TWW: TU_{20} 2.5 (based on IC_{50} of REF 1.6 converted to IC_{20} of REF 0.40 assuming slope of 1)
- SW: TU_{20} 0.34 (based on IC_{50} of REF 11.8 converted to IC_{20} of REF 2.95 assuming slope of 1)

In addition, another bacterial toxicity assay, the Microtox assay, has also been applied to different water types, with the typical range from Escher et al. (2021) reported below:

- WW: TU_{20} 0.24 – 8 (based on EC_{50} 0.48 – 17, *i.e.*, EC_{20} 0.12 – 4.25 assuming slope of 1)
- TWW: TU_{20} 0.15 – 1.33 (based on EC_{50} 3 – 27, *i.e.*, EC_{20} 0.75 – 6.75 assuming slope of 1)
- SW: TU_{20} 0.05 – 0.49 (based on EC_{50} 8.2 – 87, *i.e.*, EC_{20} 2.1 – 21.8 assuming slope of 1)

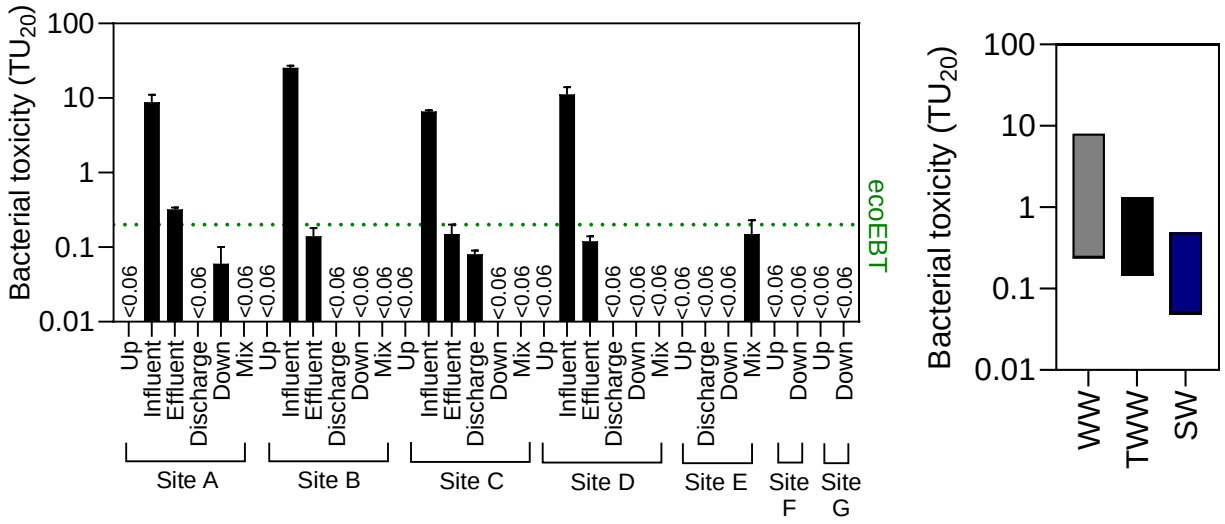

Figure 1. Toxicity of water extracts in the BLT-Screen, expressed as Toxic Unit at IC_{20} (TU_{20}), with TU_{20} plotted on a log scale. Green dotted line indicates the ecoEBT value (ecoEBT). Panel on the right indicates typical ranges for wastewater (WW), treated wastewater (TWW) and surface water (SW) based on data presented in Chapter 10 in Escher et al. (2021).

Table 3. BLT-Screen results (Toxic Unit, TU)

Short sample ID	Full sample ID	Sample type	BLT-Screen activity (TU_{20})
1_1	Site A WWTP - Influent	Influent	8.76 ± 2.35
1_2	Site A WWTP - Effluent	Effluent	0.32 ± 0.02
1_3	Site A WWTP - Up	Surface water	BDL (<0.06)
1_4	Site A WWTP - Down	Surface water	0.06 ± 0.04
1_5	Site A WWTP - Discharge	Surface water	BDL (<0.06)
1_6	Site A WWTP - Mix	Surface water	BDL (<0.06)
1_7	Ultrapure water control	Control	BDL (<0.06)
4_1	Site B WWTP - Influent	Influent	25.4 ± 1.95
4_2	Site B WWTP - Effluent	Effluent	0.14 ± 0.04
4_3	Site B WWTP - Up	Surface water	BDL (<0.06)
4_4	Site B WWTP - Down	Surface water	BDL (<0.06)
4_5	Site B WWTP - Discharge	Surface water	BDL (<0.06)
4_6	Site B WWTP - Mix	Surface water	BDL (<0.06)
2_1	Site C WWTP - Influent	Influent	6.60 ± 0.24
2_2	Site C WWTP - Effluent	Effluent	0.15 ± 0.05
2_3	Site C WWTP - Up	Surface water	BDL (<0.06)
2_4	Site C WWTP - Down	Surface water	BDL (<0.06)
2_5	Site C WWTP - Discharge	Surface water	0.08 ± 0.01
2_6	Site C WWTP - Mix	Surface water	BDL (<0.06)
2_7	Ultrapure water control	Control	BDL (<0.06)
3_1	Site D WWTP - Influent	Influent	11.1 ± 2.83
3_2	Site D WWTP - Effluent	Effluent	0.12 ± 0.02
3_3	Site D WWTP - Up	Surface water	BDL (<0.06)
3_4	Site D WWTP - Down	Surface water	BDL (<0.06)
3_5	Site D WWTP - Discharge	Surface water	BDL (<0.06)
3_6	Site D WWTP - Mix	Surface water	BDL (<0.06)

Short sample ID	Full sample ID	Sample type	BLT-Screen activity (TU_{20})
6_1	Site E - Up	Surface water	BDL (<0.06)
6_2	Site E - Down	Surface water	BDL (<0.06)
6_3	Site E - Discharge	Surface water	BDL (<0.06)
6_4	Site E - Mix	Surface water	0.15 ± 0.08
5_1	Site F - Up	Surface water	BDL (<0.06)
5_2	Site F - Down	Surface water	BDL (<0.06)
5_3	Site G - Up	Surface water	BDL (<0.06)
5_4	Site G - Down	Surface water	BDL (<0.06)

3.3 IPAM

Results: The wastewater influent samples had the greatest response in the photosynthesis inhibition assay, ranging from 0.37 to 1.26 µg DEQ/L for 2h-IPAM (Figure 2) and 0.45 to 0.97 µg DEQ/L for 24h-IPAM (Figure 3). Wastewater treatment reduced the IPAM activity, with the effect of the effluent samples ranging from 0.02 to 0.14 µg DEQ/L for 2h-IPAM and 0.03 to 0.12 µg DEQ/L for 24h-IPAM. All DEQ values for both 2h- and 24h-IPAM are provided in Table 4. The removal efficiency was similar for both 2h- and 24h-IPAM activity and ranged from 75% to 98% for 2h-IPAM and 76% to 97% for 24h-IPAM. The lowest removal for both 2h- and 24h-IPAM activity was observed at Site A WWTP.

Only the wastewater effluent from Site A exceeded the ecoEBT for 2h-IPAM activity, while the effluent from Site A and Site C exceeded the ecoEBT for 24h-IPAM activity. However, none of the wastewater effluent samples would be above the ecoEBT after 10 times dilution in the receiving waters. All surface water samples were below the ecoEBT for both 2h-IPAM and 24h-IPAM, indicating a low risk. The surface water and wastewater samples were within a similar range to previously detected for both 2h- and 24h-IPAM.

Effect based trigger values: The ecoEBT is 0.07 µg DEQ/L for the 2h-IPAM (Escher et al., 2018) and 0.09 µg DEQ/L for the 24h-IPAM. The 24h-IPAM value was not published in Escher et al. (2018), but was calculated using the same approach with single chemical data from the Swiss Ecotox Centre.

Typical range: The typical range in different water types for 2h-IPAM and 24 h-IPAM from Escher et al. (2021) are reported below:

- WW: 0.07 – 2.2 µg DEQ/L for 2h-IPAM
- TWW: 0.03 – 1.3 µg DEQ/L for 2h-IPAM and 0.07 – 0.95 µg DEQ/L for 24h-IPAM
- SW: 0.01 – 1.3 µg DEQ/L for 2h-IPAM and <0.006 – 0.52 µg DEQ/L for 24h-IPAM

Typical removal efficacy: 2h-IPAM activity is typically poorly removed by secondary WWTPs, with 28 to 52% removal reported (Escher et al., 2008; Neale et al., 2020b). Removal is often better for 24h-IPAM as more chemicals in addition to PSII herbicides can also contribute to photosynthesis inhibition after

24h. Tertiary WWTPs with ozonation show better removal of 2h-IPAM activity, with 80% removal reported (Neale et al., 2020b).

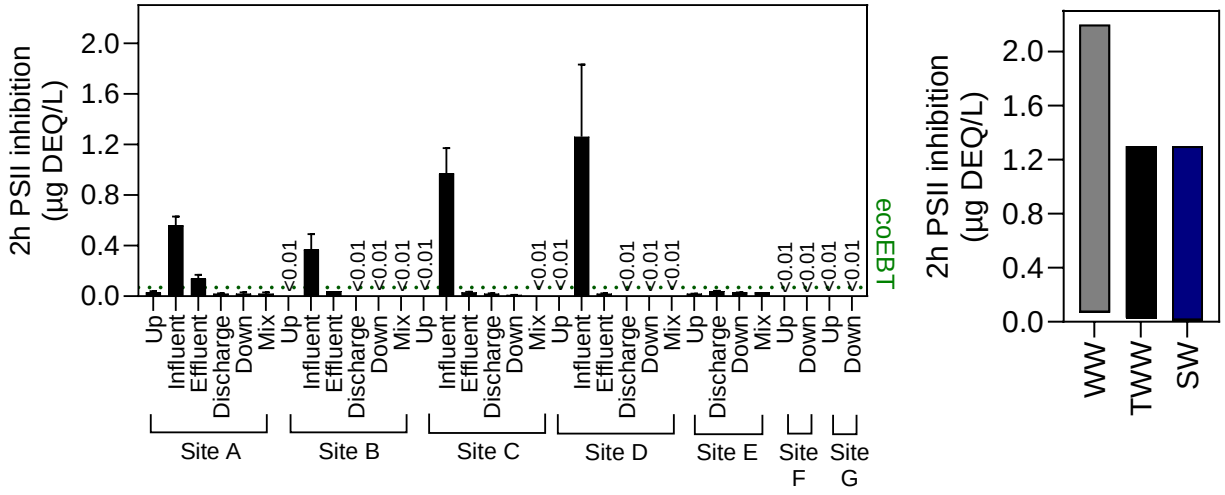

Figure 2. Photosynthesis inhibition (2h-IPAM) in the water extracts, expressed as µg Diuron Equivalent (DEQ) / L. Green dotted line indicates the ecoEBT value (ecoEBT). Panel on the right indicates typical ranges for wastewater (WW), treated wastewater (TWW) and surface water (SW) based on data presented in Chapter 10 in Escher et al. (2021).

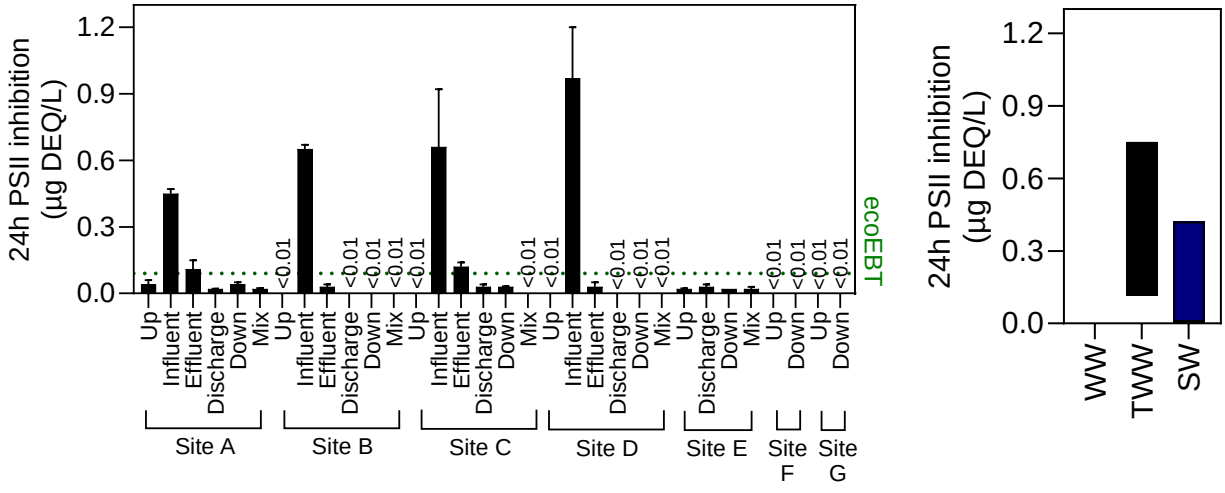

Figure 3. Photosynthesis inhibition (24h-IPAM) in the water extracts, expressed as µg Diuron Equivalent (DEQ) / L. Green dotted line indicates the ecoEBT value (ecoEBT). Panel on the right indicates typical ranges for treated wastewater (TWW) and surface water (SW) based on data presented in Chapter 10 in Escher et al. (2021).

Table 4. IPAM results (µg diuron equivalent / L)

Short sample ID	Full sample ID	Sample type	2h-IPAM response (µg DEQ/L)	24h-IPAM response (µg DEQ/L)
1_1	Site A WWTP - Influent	Influent	0.56 ± 0.07	0.45 ± 0.02

Short sample ID	Full sample ID	Sample type	2h-IPAM response (µg DEQ/L)	24h-IPAM response (µg DEQ/L)
1_2	Site A WWTP - Effluent	Effluent	0.14 ± 0.03	0.11 ± 0.04
1_3	Site A WWTP - Up	Surface water	0.03 ± 0.01	0.04 ± 0.02
1_4	Site A WWTP - Down	Surface water	0.02 ± 0.01	0.04 ± 0.01
1_5	Site A WWTP - Discharge	Surface water	0.02 ± 0.004	0.02 ± 0.002
1_6	Site A WWTP - Mix	Surface water	0.02 ± 0.01	0.02 ± 0.004
1_7	Ultrapure water control	Control	BDL (<0.01)	BDL (<0.01)
4_1	Site B WWTP - Influent	Influent	0.37 ± 0.12	0.65 ± 0.02
4_2	Site B WWTP - Effluent	Effluent	0.04 ± 0.001	0.03 ± 0.01
4_3	Site B WWTP - Up	Surface water	BDL (<0.01)	BDL (<0.01)
4_4	Site B WWTP - Down	Surface water	BDL (<0.01)	BDL (<0.01)
4_5	Site B WWTP - Discharge	Surface water	BDL (<0.01)	BDL (<0.01)
4_6	Site B WWTP - Mix	Surface water	BDL (<0.01)	BDL (<0.01)
2_1	Site C WWTP - Influent	Influent	0.97 ± 0.20	0.66 ± 0.26
2_2	Site C WWTP - Effluent	Effluent	0.03 ± 0.005	0.12 ± 0.02
2_3	Site C WWTP - Up	Surface water	BDL (<0.01)	BDL (<0.01)
2_4	Site C WWTP - Down	Surface water	0.01 ± 0.002	0.03 ± 0.003
2_5	Site C WWTP - Discharge	Surface water	0.02 ± 0.003	0.03 ± 0.01
2_6	Site C WWTP - Mix	Surface water	BDL (<0.01)	BDL (<0.01)
2_7	Ultrapure water control	Control	BDL (<0.01)	BDL (<0.01)
3_1	Site D WWTP - Influent	Influent	1.26 ± 0.57	0.97 ± 0.23
3_2	Site D WWTP - Effluent	Effluent	0.02 ± 0.004	0.03 ± 0.02
3_3	Site D WWTP - Up	Surface water	BDL (<0.01)	BDL (<0.01)
3_4	Site D WWTP - Down	Surface water	BDL (<0.01)	BDL (<0.01)
3_5	Site D WWTP - Discharge	Surface water	BDL (<0.01)	BDL (<0.01)
3_6	Site D WWTP - Mix	Surface water	BDL (<0.01)	BDL (<0.01)
6_1	Site E - Up	Surface water	0.02 ± 0.001	0.02 ± 0.003
6_2	Site E - Down	Surface water	0.03 ± 0.001	0.02 ± 0.000
6_3	Site E - Discharge	Surface water	0.04 ± 0.002	0.03 ± 0.01
6_4	Site E - Mix	Surface water	0.03 ± 0.000	0.02 ± 0.01
5_1	Site F - Up	Surface water	BDL (<0.01)	BDL (<0.01)
5_2	Site F - Down	Surface water	BDL (<0.01)	BDL (<0.01)
5_3	Site G - Up	Surface water	BDL (<0.01)	BDL (<0.01)
5_4	Site G - Down	Surface water	BDL (<0.01)	BDL (<0.01)

3.4 ER-GeneBLAzer

Results: The wastewater influent samples had the greatest response in the ER-GeneBLAzer assay, with estrogenic activity ranging from 37.9 to 53.7 ng EEQ/L (Figure 4 and Table 5). Wastewater treatment reduced the estrogenic activity to 1.87 to 38.2 EEQ/L. No anti-estrogenic activity was observed in any sample (Table 5). Between 88 to 95% removal of estrogenic activity was observed for the Sites B, C and D WWTPs, which is within the typical range reported in the literature. In contrast, the Site A WWTP only removed 29% of estrogenic activity.

All surface water samples from Site A, the downstream (2_4) and discharge (2_5) samples from Site C, the discharge (3_5) and mix (3_6) samples from Site D and the downstream (6_2), discharge (6_3) and mix (6_4) samples from Site E exceeded the ecoEBT of 0.34 ng EEQ/L. Exceedance of the ecoEBT indicates a possible risk, though most surface water samples were less than 5 times higher than the ecoEBT. An exceedance of the ecoEBT warrants further investigation, with the steps to be taken depending on the magnitude of exceedance. This is described in detail in Neale et al. (2023b). Briefly, the first step would be to collect another sample from the same site and retest to confirm that the exceedance was not an isolated occurrence. If the second sample also exceeds the ecoEBT, then targeted chemical analysis of known potent chemicals is recommended for assays where few potent chemicals explain most of the effect, such as ER-GeneBLAzer. The bioassay response can be compared to the predicted response based on detected chemicals. If the two values agree, then it can be concluded that the known chemicals explain the effect and these can be compared to chemical guideline values. Further action is required if known chemicals cannot explain the observed effect and the effect is over 10 times higher than the ecoEBT This could include using effect-directed analysis (EDA) to identify causative chemicals. If EDA cannot identify the causative chemicals additional steps in consultation with relevant regulatory bodies is required. This could include optimising the treatment process to remove the bioassay response.

The discharge (3_5) from Site D presented the highest risk, with the detected estrogenic activity over 25 times higher than the ecoEBT. Further, the treated effluent was above the ecoEBT for all four WWTPs, though the effluent from Sites B and D would be below the ecoEBT after 10 times dilution into receiving waters. In contrast, the effluent from Site A WWTP would need to be diluted over 100 times in the receiving environment to be below the ecoEBT.

The observed effect in wastewater influent was higher than reported in the literature, though few studies have applied the ER-GeneBLAzer assay to wastewater influent. Estrogenic activity in treated wastewater and surface water was within the ranged reported in the literature.

Effect based trigger values: The ER-GeneBLAzer ecoEBT is 0.34 ng EEQ/L (Escher et al., 2018).

Typical range: The typical range in different water types for ER-GeneBLAzer from Escher et al. (2021) are reported below:

Agonist mode

- WW: 11 – 24 ng EEQ/L
- TWW: 0.03 – 151 ng EEQ/L
- SW: 0.005 – 39 ng EEQ/L

Antagonist mode

- WW: cytotoxic
- TWW: <2177 µg TMXEQ/L, cytotoxic
- SW: <1–2.7 µg TMXEQ/L, cytotoxic

Typical removal efficacy: Estrogenic activity is usually well removed by secondary and tertiary WWTPs, with between 80 to >99% removal efficacy reported (summarised in Escher et al. (2021)). Lower removal, 56 to 57%, was reported for primary WWTPs (Neale et al., 2020b).

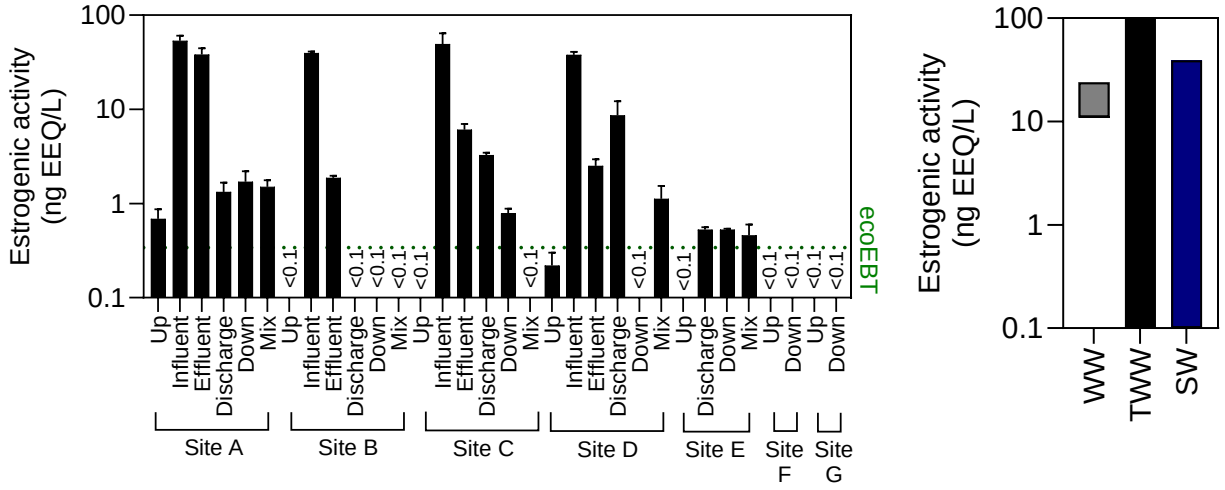

Figure 4. Estrogenic activity in the water extracts, expressed as ng Estradiol Equivalent (EEQ) / L, with EEQ plotted on a log scale. Green dotted line indicates the ecoEBT value (ecoEBT). Panel on the right indicates typical ranges for treated wastewater (TWW) and surface water (SW) based on data presented in Chapter 10 in Escher et al. (2021).

Table 5. ER-GeneBLAzer results in agonist (ng estradiol equivalent / L) and antagonist modes (ng TMXEQ/L).

Short sample ID	Full sample ID	Sample type	Estrogenicity (ng EEQ/L)	Anti-estrogenicity (ng TMXEQ/L)
1_1	Site A WWTP - Influent	Influent	53.7 ± 7.08	BDL (<3085*)
1_2	Site A WWTP - Effluent	Effluent	38.2 ± 6.24	BDL (<193)
1_3	Site A WWTP - Up	Surface water	0.69 ± 0.18	BDL (<193)
1_4	Site A WWTP - Down	Surface water	1.71 ± 0.49	BDL (<193)
1_5	Site A WWTP - Discharge	Surface water	1.33 ± 0.34	BDL (<193)
1_6	Site A WWTP - Mix	Surface water	1.51 ± 0.26	BDL (<193)
1_7	Ultrapure water control	Control	BDL (<0.1)	BDL (<193)
4_1	Site B WWTP - Influent	Influent	39.7 ± 1.28	BDL (<3085*)
4_2	Site B WWTP - Effluent	Effluent	1.87 ± 0.10	BDL (<193)
4_3	Site B WWTP - Up	Surface water	BDL (<0.1)	BDL (<193)
4_4	Site B WWTP - Down	Surface water	BDL (<0.1)	BDL (<193)
4_5	Site B WWTP - Discharge	Surface water	BDL (<0.1)	BDL (<193)

Short sample ID	Full sample ID	Sample type	Estrogenicity (ng EEQ/L)	Anti-estrogenicity (ng TMXEQ/L)
4_6	Site B WWTP - Mix	Surface water	BDL (<0.1)	BDL (<193)
2_1	Site C WWTP - Influent	Influent	49.4 ± 14.7	BDL (<12323*)
2_2	Site C WWTP - Effluent	Effluent	6.09 ± 0.89	BDL (<193)
2_3	Site C WWTP - Up	Surface water	BDL (<0.1)	BDL (<193)
2_4	Site C WWTP - Down	Surface water	0.79 ± 0.09	BDL (<193)
2_5	Site C WWTP - Discharge	Surface water	3.26 ± 0.20	BDL (<193)
2_6	Site C WWTP - Mix	Surface water	BDL (<0.1)	BDL (<193)
2_7	Ultrapure water control	Control	BDL (<0.1)	BDL (<193)
3_1	Site D WWTP - Influent	Influent	37.9 ± 2.77	BDL: (<3085*)
3_2	Site D WWTP - Effluent	Effluent	2.52 ± 0.43	BDL (<193)
3_3	Site D WWTP - Up	Surface water	0.22 ± 0.08	BDL (<193)
3_4	Site D WWTP - Down	Surface water	BDL (<0.1)	BDL (<193)
3_5	Site D WWTP - Discharge	Surface water	8.67 ± 3.54	BDL (<193)
3_6	Site D WWTP - Mix	Surface water	1.12 ± 0.42	BDL (<193)
6_1	Site E - Up	Surface water	BDL (<0.1)	BDL (<193)
6_2	Site E - Down	Surface water	0.53 ± 0.01	BDL (<193)
6_3	Site E - Discharge	Surface water	0.53 ± 0.03	BDL (<193)
6_4	Site E - Mix	Surface water	0.46 ± 0.14	BDL (<193)
5_1	Site F - Up	Surface water	BDL (<0.1)	BDL (<193)
5_2	Site F - Down	Surface water	BDL (<0.1)	BDL (<193)
5_3	Site G - Up	Surface water	BDL (<0.1)	BDL (<193)
5_4	Site G - Down	Surface water	BDL (<0.1)	BDL (<193)

*Higher detection limit due to cytotoxicity

3.5 ARE-GeneBLAzer

Results: Oxidative stress response was detected in all wastewater samples as well as 63% of the surface water samples, with the response in the remaining surface water samples below detection limit of 193 µg DDVPEQ/L (Table 6). The highest response was observed in the wastewater influents, with DDVPEQ ranging from 6,011 to 21,952 µg DDVPEQ/L (Figure 5). Wastewater treatment reduced the oxidative stress response, with between 79 to 96% removal at the studied WWTPs.

All surface water samples from Site E, the upstream (1_3) sample from Site A and the upstream (2_3), downstream (2_4) and discharge (2_5) samples from Site C exceeded the ecoEBT of 392 µg DDVPEQ/L. Exceedance of the ecoEBT suggests a potential risk, though the measured DDVPEQ were only up to 2.5 times higher than the ecoEBT. In contrast, all surface water samples from Site B, Site D and the control sites (Site F and Site G) were below the ecoEBT, indicating a low risk. Further, none of the wastewater effluent samples would be above the ecoEBT after 10 times dilution in the receiving

waters. The observed effect in wastewater influent and effluent were higher than typically reported in the literature, while the effect in surface water was generally within the range reported in the literature.

Effect based trigger values: The ARE-GeneBLAzer ecoEBT is 392 µg DDVPEQ/L (Escher et al., 2018).

Typical range: The typical range in different water types for ARE-GeneBLAzer from Escher et al. (2021) are reported below. The results are usually reported as $EC_{IR1.5}$ values, so these have also been reported. As ARE-GeneBLAzer has only been run in TWW and SW, the typical range in WW is reported for the AREc32 assay, which is also indicative of oxidative stress.

- WW*: 362 – 6080 µg DDVPEQ/L ($EC_{IR1.5}$ 0.28–4.7 REF)
- TWW: 227 – 434 µg/L DDVPEQ/L ($EC_{IR1.5}$ 8.9 – 17 REF)
- SW: <8 – 560 µg/L DDVPEQ/L ($EC_{IR1.5}$ 6.9 - >490 REF)

*results for AREc32 assay.

Typical removal efficacy: Between 39 to 85% removal of oxidative stress response was reported for secondary and tertiary WWTPs (Neale et al., 2020b; Nivala et al., 2018; Völker et al., 2017), with lower removal efficacy, -35 to 23%, for primary WWTPs (Neale et al., 2020b).

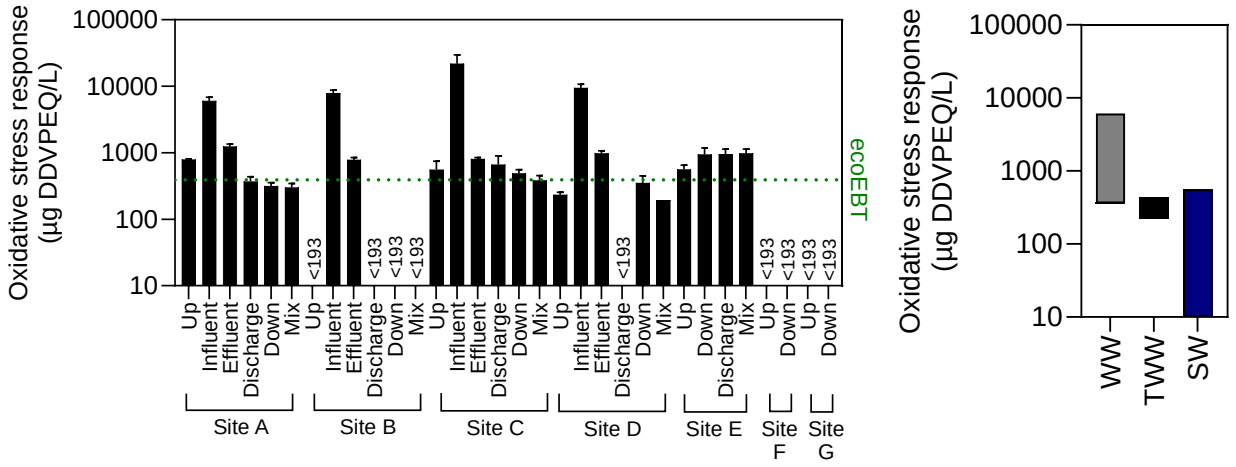

Figure 5. Oxidative stress activity in water extracts, expressed as µg Dichlorvos Equivalent (DDVPEQ) / L, with DDVPEQ plotted on a log scale. Green dotted line indicates the ecoEBT value (ecoEBT). Panel on the right indicates typical ranges for wastewater (WW), treated wastewater (TWW) and surface water (SW) based on data presented in Chapter 10 in Escher et al. (2021).

Table 6. ARE-GeneBLAzer results (µg dichlorvos equivalent / L))

Short sample ID	Full sample ID	Sample type	ARE-GeneBLAzer response (µg DDVPEQ/L)
1_1	Site A WWTP - Influent	Influent	6011 ± 842
1_2	Site A WWTP - Effluent	Effluent	1242 ± 117

Short sample ID	Full sample ID	Sample type	ARE-GeneBLAzer response (µg DDVPEQ/L)
1_3	Site A WWTP - Up	Surface water	794 ± 15
1_4	Site A WWTP - Down	Surface water	317 ± 37
1_5	Site A WWTP - Discharge	Surface water	370 ± 64
1_6	Site A WWTP - Mix	Surface water	302 ± 43
1_7	Ultrapure water control	Control	BDL (<193)
4_1	Site B WWTP - Influent	Influent	7891 ± 828
4_2	Site B WWTP - Effluent	Effluent	780 ± 68
4_3	Site B WWTP - Up	Surface water	BDL (<193)
4_4	Site B WWTP - Down	Surface water	BDL (<193)
4_5	Site B WWTP - Discharge	Surface water	BDL (<193)
4_6	Site B WWTP - Mix	Surface water	BDL (<193)
2_1	Site C WWTP - Influent	Influent	21952 ± 7794
2_2	Site C WWTP - Effluent	Effluent	809 ± 35
2_3	Site C WWTP - Up	Surface water	554 ± 191
2_4	Site C WWTP - Down	Surface water	489 ± 68
2_5	Site C WWTP - Discharge	Surface water	666 ± 235
2_6	Site C WWTP - Mix	Surface water	385 ± 69
2_7	Ultrapure water control	Control	BDL (<193)
3_1	Site D WWTP - Influent	Influent	9409 ± 1318
3_2	Site D WWTP - Effluent	Effluent	984 ± 92
3_3	Site D WWTP - Up	Surface water	233 ± 21
3_4	Site D WWTP - Down	Surface water	351 ± 99
3_5	Site D WWTP - Discharge	Surface water	BDL (<193)
3_6	Site D WWTP - Mix	Surface water	194 ± 138
6_1	Site E - Up	Surface water	564 ± 89
6_2	Site E - Down	Surface water	944 ± 234
6_3	Site E - Discharge	Surface water	955 ± 182
6_4	Site E - Mix	Surface water	978 ± 154
5_1	Site F - Up	Surface water	BDL (<193)
5_2	Site F - Down	Surface water	BDL (<193)
5_3	Site G - Up	Surface water	BDL (<193)
5_4	Site G - Down	Surface water	BDL (<193)

3.6 AhR-CAFLUX

Results: AhR activity was detected in most samples, including one of the ultrapure water controls, though at levels close to the detection limit of 1.03 µg DEQ/L or 18 pg TCDD/L (Table 7). As noted above, results for AhR-CAFLUX were expressed as both DEQ and TCDDEQ. While results are typically expressed as TCDDEQ, concerns about working with highly toxic TCDD meant that diuron is now used as the assay reference compound. In addition to dioxin-like chemicals, many chemicals, including pesticides and industrial chemicals, can activate AhR (Neale et al., 2020a) and these chemical may be introduced into controls through sample collection, processing and extraction. As a result of activity in one of the controls, blank corrected DEQ or TCDDEQ values are presented in this section.

The highest AhR activity was observed in sample 1_2, wastewater effluent from Site A WWTP, with 41 µg DEQ/L or 713 pg TCDDEQ/L. With the exception of this site, wastewater treatment reduced AhR activity, with between 50 and 91% removal for Site B, C and D WWTPs.

Focusing on the effect reported as TCDDEQ/L, the upstream (1_3), downstream (1_4) and discharge (1_5) samples from Site A slightly exceeded the ecoEBT of 50 pg TCDDEQ/L (<1.4 times higher) (Figure 6). Further, only wastewater effluent from Site A would be above the ecoEBT after 10 times dilution in the receiving waters. Exceedance of the ecoEBT suggests a potential risk. In contrast, all surface water samples above the assay limit of detection from Site C and Site E were below the ecoEBT, indicating a low risk. Further, all surface water samples from Site B, Site D and the control sites (Site F and Site G) were below the assay limit of detection. The observed effect in wastewater effluent and surface water were within the range reported in the literature, while the effect in wastewater influent was lower than reported, though few studies have applied this assay to wastewater influent.

Effect based trigger values: The AhR-CAFLUX ecoEBT is 50 pg TCDDEQ/L (van der Oost et al., 2017).

Typical range: The typical range in different water types for AhR-CAFLUX from Escher et al. (2021) are reported below:

- WW: 1100 – 1800 pg TCDDEQ/L
- TWW: 7 – 1200 pg TCDDEQ/L
- SW: 10 – 190 pg TCDDEQ/L

Typical removal efficacy: Between -15% to 90% removal of AhR activity was reported for secondary and tertiary WWTPs (Jálová et al., 2013; Neale et al., 2020b; Nivala et al., 2018). Low removal, 8 to 18%, was reported for primary WWTPs (Neale et al., 2020b).

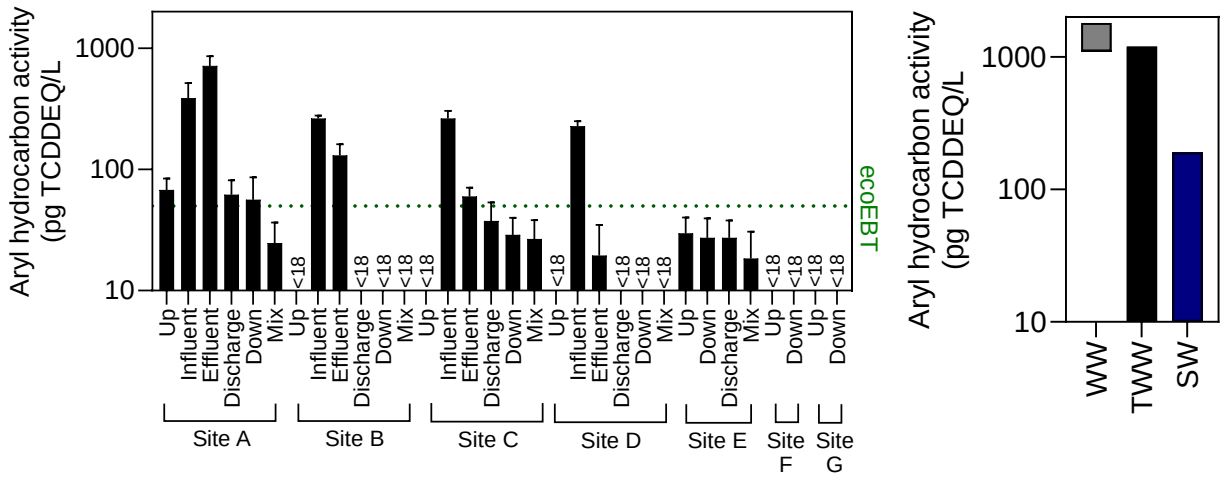

Figure 6. Aryl hydrocarbon receptor (AhR) activity in water extracts, expressed as pg TCDD Equivalent (TCDDEQ) / L, with TCDDEQ plotted on a log scale. TCDDEQ values are blank corrected. Green dotted line indicates the ecoEBT value (ecoEBT). Panel on the right indicates typical ranges for wastewater (WW), treated wastewater (TWW) and surface water (SW) based on data presented in Chapter 10 in Escher et al. (2021).

Table 7. AhR-CAFLUX results (µg DEQ equivalent / L and pg TCDD equivalent / L). All results are blank corrected

Short sample ID	Full sample ID	Sample type	Aryl hydrocarbon activity (µg DEQ/L)	Aryl hydrocarbon activity (pg TCDDEQ/L)
1_1	Site A WWTP – Influent	Influent	22.3 ± 7.33	387 ± 128
1_2	Site A WWTP – Effluent	Effluent	41.0 ± 8.43	713 ± 147
1_3	Site A WWTP – Up	Surface water	3.89 ± 1.18	67.7 ± 16.3
1_4	Site A WWTP – Down	Surface water	3.23 ± 1.73	56.3 ± 30.0
1_5	Site A WWTP – Discharge	Surface water	3.55 ± 1.12	61.7 ± 19.5
1_6	Site A WWTP – Mix	Surface water	1.41 ± 0.68	24.6 ± 11.8
1_7	Ultrapure water control	Control	BDL (<1)	BDL (<18)
4_1	Site B WWTP – Influent	Influent	15.1 ± 0.92	263 ± 15.9
4_2	Site B WWTP – Effluent	Effluent	7.52 ± 1.78	131 ± 30.9
4_3	Site B WWTP – Up	Surface water	BDL (<1)	BDL (<18)
4_4	Site B WWTP – Down	Surface water	BDL (<1)	BDL (<18)
4_5	Site B WWTP – Discharge	Surface water	BDL (<1)	BDL (<18)
4_6	Site B WWTP – Mix	Surface water	BDL (<1)	BDL (<18)
2_1	Site C WWTP – Influent	Influent	15.1 ± 2.38	262 ± 41.4
2_2	Site C WWTP – Effluent	Effluent	3.43 ± 0.62	59.8 ± 10.9
2_3	Site C WWTP – Up	Surface water	BDL (<1)	BDL (<18)
2_4	Site C WWTP – Down	Surface water	1.65 ± 0.63	28.8 ± 11.0
2_5	Site C WWTP – Discharge	Surface water	2.15 ± 0.93	37.5 ± 16.1
2_6	Site C WWTP – Mix	Surface water	1.53 ± 0.66	26.7 ± 11.5
2_7	Ultrapure water control	Control	BDL (<1)	BDL (<18)
3_1	Site D WWTP – Influent	Influent	13.1 ± 1.36	227 ± 23.7
3_2	Site D WWTP – Effluent	Effluent	1.12 ± 0.89	19.4 ± 15.4
3_3	Site D WWTP – Up	Surface water	BDL (<1)	BDL (<18)
3_4	Site D WWTP – Down	Surface water	BDL (<1)	BDL (<18)

Short sample ID	Full sample ID	Sample type	Aryl hydrocarbon activity (µg DEQ/L)	Aryl hydrocarbon activity (pg TCDDEQ/L)
3_5	Site D WWTP – Discharge	Surface water	BDL (<1)	BDL (<18)
3_6	Site D WWTP – Mix	Surface water	BDL (<1)	BDL (<18)
6_1	Site E – Up	Surface water	1.70 ± 0.60	29.6 ± 10.4
6_2	Site E – Down	Surface water	1.57 ± 0.70	27.3 ± 12.2
6_3	Site E – Discharge	Surface water	1.57 ± 0.61	27.3 ± 10.7
6_4	Site E – Mix	Surface water	1.06 ± 0.70	18.4 ± 12.2
5_1	Site F - Up	Surface water	BDL (<1)	BDL (<18)
5_2	Site F - Down	Surface water	BDL (<1)	BDL (<18)
5_3	Site G - Up	Surface water	BDL (<1)	BDL (<18)
5_4	Site G - Down	Surface water	BDL (<1)	BDL (<18)

3.7 HiTMiN

Results: All samples were below the assay limit of detection for genotoxicity (<0.05 GTU) in the HiTMiN assay. Further, some of the samples, specifically all wastewater influent samples and wastewater effluent from Sites B and D, were cytotoxic at higher concentrations, resulting in a higher detection limit. There is a narrow window between genotoxicity and cytotoxicity, so it is possible that genotoxic compounds were contributing to the observed cytotoxicity.

Effect based trigger values: There are currently no ecoEBT for genotoxicity.

Typical range: The HiTMiN assay has only recently been applied to SW passive sampler extracts from New Zealand (Leusch et al., 2024). The effect in the polyethylene samplers (targeting non-polar chemicals) ranged from <0.0018 to 0.0060 GTU and the effect in ChemCatcher passive sampler extracts (targeting polar chemicals) ranged from <0.12 to 0.13 GTU. There are also no removal efficacy data to date.

Table 8. HiTMiN results in genotoxic unit (GTU)

Short sample ID	Full sample ID	Sample type	Genotoxicity (GTU)
1_1	Site A WWTP – Influent	Influent	BDL (<0.8)*
1_2	Site A WWTP – Effluent	Effluent	BDL (<0.05)
1_3	Site A WWTP – Up	Surface water	BDL (<0.2)*
1_4	Site A WWTP – Down	Surface water	BDL (<0.05)
1_5	Site A WWTP – Discharge	Surface water	BDL (<0.05)
1_6	Site A WWTP – Mix	Surface water	BDL (<0.05)
1_7	Ultrapure water control	Control	BDL (<0.05)
4_1	Site B WWTP - Influent	Influent	BDL (<0.8)*
4_2	Site B WWTP - Effluent	Effluent	BDL (<0.2)*
4_3	Site B WWTP - Up	Surface water	BDL (<0.05)
4_4	Site B WWTP - Down	Surface water	BDL (<0.05)
4_5	Site B WWTP - Discharge	Surface water	BDL (<0.05)

Short sample ID	Full sample ID	Sample type	Genotoxicity (GTU)
4_6	Site B WWTP - Mix	Surface water	BDL (<0.05)
2_1	Site C WWTP – Influent	Influent	Cytotoxic
2_2	Site C WWTP – Effluent	Effluent	BDL (<0.05)
2_3	Site C WWTP - Up	Surface water	BDL (<0.05)
2_4	Site C WWTP - Down	Surface water	BDL (<0.05)
2_5	Site C WWTP - Discharge	Surface water	BDL (<0.05)
2_6	Site C WWTP - Mix	Surface water	BDL (<0.05)
2_7	Ultrapure water control	Control	BDL (<0.05)
3_1	Site D WWTP - Influent	Influent	BDL (<0.8)*
3_2	Site D WWTP - Effluent	Effluent	BDL (<0.2)*
3_3	Site D WWTP - Up	Surface water	BDL (<0.05)
3_4	Site D WWTP - Down	Surface water	BDL (<0.05)
3_5	Site D WWTP - Discharge	Surface water	BDL (<0.05)
3_6	Site D WWTP - Mix	Surface water	BDL (<0.05)
6_1	Site E – Up	Surface water	BDL (<0.05)
6_2	Site E - Down	Surface water	BDL (<0.2)*
6_3	Site E - Discharge	Surface water	BDL (<0.2)*
6_4	Site E – Mix	Surface water	BDL (<0.2)*
5_1	Site F - Up	Surface water	BDL (<0.05)
5_2	Site F - Down	Surface water	BDL (<0.05)
5_3	Site G - Up	Surface water	BDL (<0.05)
5_4	Site G - Down	Surface water	BDL (<0.05)

*Higher detection limit due to cytotoxicity

4 Conclusions

The project applied a suite of *in vitro* bioassays to evaluate bioactivity in the influent and effluent of four WWTPs, as well as creeks and streams upstream and downstream of the WWTPs. All wastewater influent and effluent samples had a response in the studied bioassays, except for the HiTMiN assay, which is indicative of genotoxicity. Overall, the data show that the WWTPs can remove the majority of bioactive chemicals, with most WWTPs achieving over 80% removal of bacterial toxicity, photosynthesis inhibition, estrogenic activity and oxidative stress response. However, lower removal efficiency was consistently observed for the Site A WWTP, with particularly poor removal of estrogenic activity and AhR activity.

The water quality upstream and downstream of the WWTPs was mostly good for the majority of studied endpoints, with the observed effects similar to previously reported activity for surface water globally. Few of the surface water samples were above the assay detection limit in the BLT-Screen, while photosynthesis inhibition was low in all surface water samples. All surface water samples were below their respective EBTs for bacterial toxicity and photosynthesis inhibition, indicating a low risk.

Eleven of the surface water samples had a response in the activation of AhR assay, with around 27% of surface water samples with AhR activity exceeding the ecoEBT. Further, 53% of surface water samples that had a response in the oxidative stress assay exceeded the ecoEBT for oxidative stress response. While this indicates a possibly risk, the observed effect was only up to 1.4 and 2.5 times higher than the ecoEBT for activation of AhR and oxidative stress response, respectively.

In contrast, all but one of the surface water samples that had a response in ER-GeneBLAzer exceeded the ecoEBT for estrogenic activity, with the observed effect up to 25 times higher than the ecoEBT at one site, suggesting a higher risk.

The results presented represent a snapshot in time, with a single sample taken for each WWTP. Therefore, monitoring over a longer period of time, particularly for estrogenic activity, is recommended. If the effect in the surface water is consistently found to be over 10× higher than the ecoEBT, further investigation would be warranted, including *in situ* assessment for signs of adverse ecological effects (such as feminisation of fish) and optimisation of treatment processes to reduce the estrogenic activity.

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Appendix B – Data appendices

Table S1. Summary statistics for emerging contaminants detected at each sampling point. Where a chemical was not detected in any samples at a sampling point, it was excluded from this table. SD = standard deviation; Min = minimum; Max = Maximum; LOR = limit of reporting.

Analyte group	Analyte	Sampling point	Samples (n)	Detections (n)	Mean (µg/L)	SD (µg/L)	Min (µg/L)	Max (µg/L)	LOR (µg/L)
Artificial Sweeteners	Acesulfame K	Upstream	5	3	0.094	0.16	0	0.37	0.005
	Acesulfame K	Influent	4	4	7.35	8.61	1.3	20	0.005
	Acesulfame K	Effluent	4	4	0.23	0.22	0.046	0.5	0.005
	Acesulfame K	Discharge	5	3	0.14	0.18	0	0.44	0.005
	Acesulfame K	Downstream	5	3	0.057	0.060	0	0.14	0.005
	Acesulfame K	Further downstream	5	3	0.061	0.087	0	0.21	0.005
	Cyclamate	Upstream	5	1	0.0018	0.0040	0	0.009	0.005
	Cyclamate	Influent	4	4	1.09	0.96	0.43	2.5	0.005
	Cyclamate	Effluent	4	4	0.054	0.045	0.02	0.12	0.005
	Cyclamate	Discharge	5	1	0.011	0.024	0	0.053	0.005
	Cyclamate	Downstream	5	1	0.012	0.027	0	0.061	0.005
	Cyclamate	Further downstream	5	1	0.019	0.042	0	0.093	0.005
	Saccharin	Upstream	5	3	0.013	0.016	0	0.039	0.005
	Saccharin	Influent	4	4	5.13	2.94	2.6	8.1	0.005
	Saccharin	Effluent	4	4	0.34	0.31	0.091	0.75	0.005
	Saccharin	Discharge	5	3	0.053	0.099	0	0.23	0.005
	Saccharin	Downstream	5	3	0.016	0.020	0	0.047	0.005
	Saccharin	Further downstream	5	3	0.0078	0.0091	0	0.022	0.005
	Sucralose	Upstream	5	4	0.061	0.095	0	0.23	0.005
	Sucralose	Influent	4	4	24.75	6.13	17	32	0.005
	Sucralose	Effluent	4	4	28	5.48	21	34	0.005
	Sucralose	Discharge	5	5	2.97	3.82	0.009	7.2	0.005
	Sucralose	Downstream	5	5	3.43	4.34	0.008	8.8	0.005
	Sucralose	Further downstream	5	5	2.02	2.84	0.011	6.7	0.005
EDCs	17a-Ethinylestradiol	Discharge	5	1	0.0006	0.0013	0	0.003	0.002
	17a-Ethinylestradiol	Downstream	5	1	0.0006	0.0013	0	0.003	0.002

	17a-Ethinylestradiol	Further downstream	5	1	0.0006	0.0013	0	0.003	0.002
	Androstenedione	Influent	4	2	0.013	0.016	0	0.034	0.005
	Androstenedione	Effluent	4	1	0.0018	0.0035	0	0.007	0.005
	Androsterone	Influent	4	2	0.27	0.31	0	0.55	0.005
	BHT	Influent	4	2	0.5	0.81	0	1.7	0.002
	BHT	Effluent	4	1	0.14	0.27	0	0.54	0.002
	Bisphenol A	Influent	4	4	0.095	0.037	0.057	0.14	0.002
	Bisphenol A	Effluent	4	4	0.023	0.017	0.009	0.048	0.002
	Estriol	Influent	4	4	0.13	0.043	0.078	0.18	0.002
	Estrone	Influent	4	4	0.017	0.017	0.005	0.042	0.001
	Estrone	Effluent	4	4	0.0098	0.0075	0.005	0.021	0.001
	Etiocholanolone	Influent	4	4	0.69	0.51	0.21	1.3	0.005
	Nonylphenol	Influent	4	2	6.08	11.30	0	23	0.05
	Nonylphenol	Effluent	4	2	6.13	11.92	0	24	0.05
	Nonylphenol	Discharge	5	1	0.96	2.15	0	4.8	0.05
	Nonylphenol	Downstream	5	1	0.24	0.54	0	1.2	0.05
	Nonylphenol	Further downstream	5	1	0.11	0.24	0	0.54	0.05
	Testosterone	Influent	4	3	0.038	0.035	0	0.069	0.005
	tert-octyl phenol	Influent	4	2	0.12	0.21	0	0.43	0.002
	tert-octyl phenol	Effluent	4	2	0.14	0.20	0	0.42	0.002
	tert-octyl phenol	Discharge	5	1	0.020	0.044	0	0.098	0.002
Pesticides	Atrazine	Discharge	5	1	0.002	0.0045	0	0.01	0.01
	Azoxystrobin	Influent	4	1	0.005	0.01	0	0.02	0.01
	Benalaxyl	Influent	4	2	0.023	0.033	0	0.07	0.01
	Carbendazim	Influent	4	1	0.005	0.01	0	0.02	0.01
	Carbendazim	Effluent	4	1	0.005	0.01	0	0.02	0.01
	Cyromazine	Influent	4	1	0.035	0.07	0	0.14	0.01
	Cyromazine	Effluent	4	1	0.01	0.02	0	0.04	0.01
	Deet	Upstream	5	2	0.008	0.013	0	0.03	0.01

Deet	Influent	4	4	1.93	0.98	1.1	3.2	0.01
Deet	Effluent	4	4	0.18	0.15	0.03	0.36	0.01
Deet	Discharge	5	3	0.028	0.037	0	0.09	0.01
Deet	Downstream	5	3	0.028	0.037	0	0.09	0.01
Deet	Further downstream	5	3	0.026	0.037	0	0.09	0.01
Desethyl Atrazine	Upstream	5	1	0.008	0.018	0	0.04	0.01
Desethyl Atrazine	Influent	4	1	0.01	0.02	0	0.04	0.01
Desethyl Atrazine	Effluent	4	1	0.0025	0.005	0	0.01	0.01
Desethyl Atrazine	Discharge	5	1	0.006	0.013	0	0.03	0.01
Desethyl Atrazine	Downstream	5	1	0.002	0.0045	0	0.01	0.01
Desethyl Atrazine	Further downstream	5	1	0.002	0.0045	0	0.01	0.01
Diazinon	Influent	4	1	0.0125	0.025	0	0.05	0.01
Diflufenican	Influent	4	1	0.01	0.02	0	0.04	0.01
Diflufenican	Effluent	4	1	0.0025	0.005	0	0.01	0.01
Diuron	Upstream	5	2	0.008	0.011	0	0.02	0.01
Diuron	Influent	4	4	0.043	0.021	0.02	0.06	0.01
Diuron	Effluent	4	4	0.058	0.041	0.01	0.11	0.01
Diuron	Discharge	5	3	0.014	0.017	0	0.04	0.01
Diuron	Downstream	5	3	0.014	0.017	0	0.04	0.01
Diuron	Further downstream	5	2	0.01	0.017	0	0.04	0.01
Dmst	Influent	4	1	0.0075	0.015	0	0.03	0.01
Epoxiconazole	Effluent	4	1	0.005	0.01	0	0.02	0.01
Fenhexamid	Influent	4	1	0.0075	0.015	0	0.03	0.01
Fenhexamid	Effluent	4	1	0.0025	0.005	0	0.01	0.01
Fludioxonil	Upstream	5	1	0.002	0.0045	0	0.01	0.01
Flutriafol	Influent	4	1	0.005	0.01	0	0.02	0.01
Flutriafol	Effluent	4	1	0.005	0.01	0	0.02	0.01
Flutriafol	Discharge	5	1	0.004	0.0089	0	0.02	0.01
Flutriafol	Downstream	5	1	0.002	0.0045	0	0.01	0.01

Flutriafol	Further downstream	5	1	0.002	0.0045	0	0.01	0.01
Imidacloprid	Upstream	5	1	0.002	0.0045	0	0.01	0.01
Imidacloprid	Influent	4	4	0.045	0.0058	0.04	0.05	0.01
Imidacloprid	Effluent	4	4	0.048	0.028	0.02	0.08	0.01
Imidacloprid	Discharge	5	1	0.004	0.0089	0	0.02	0.01
Imidacloprid	Downstream	5	1	0.006	0.013	0	0.03	0.01
Imidacloprid	Further downstream	5	1	0.002	0.0045	0	0.01	0.01
MGK-264	Influent	4	3	0.023	0.021	0	0.05	0.01
Metazachlor	Effluent	4	1	0.005	0.01	0	0.02	0.01
Methamidophos	Effluent	4	2	0.0075	0.0096	0	0.02	0.01
Metolachlor	Upstream	5	1	0.002	0.0045	0	0.01	0.01
Metsulfuron-methyl	Upstream	5	2	0.032	0.049	0	0.11	0.01
Metsulfuron-methyl	Influent	4	3	0.0075	0.005	0	0.01	0.01
Metsulfuron-methyl	Effluent	4	1	0.0025	0.005	0	0.01	0.01
Metsulfuron-methyl	Discharge	5	3	0.036	0.042	0	0.1	0.01
Metsulfuron-methyl	Downstream	5	2	0.012	0.022	0	0.05	0.01
Metsulfuron-methyl	Further downstream	5	2	0.006	0.0089	0	0.02	0.01
Pentachloronitrobenzene (PCNB)	Upstream	5	1	0.002	0.0045	0	0.01	0.01
Permethrin, (1R)-cis	Influent	4	4	0.018	0.015	0.01	0.04	0.01
Permethrin, (1R)-trans	Influent	4	2	0.0075	0.0096	0	0.02	0.01
Piperonyl butoxide	Influent	4	4	0.10	0.10	0.04	0.25	0.01
Prometryn	Discharge	4	1	0.0025	0.005	0	0.01	0.01
Prometryn	Downstream	4	1	0.0025	0.005	0	0.01	0.01
Prometryn	Further downstream	4	1	0.0025	0.005	0	0.01	0.01
Pronamide	Upstream	5	1	0.002	0.0045	0	0.01	0.01
Propham	Influent	4	1	0.11	0.23	0	0.45	0.01
Propiconazole	Influent	4	3	0.025	0.024	0	0.05	0.01
Propiconazole	Effluent	4	2	0.02	0.023	0	0.04	0.01

	Propiconazole	Discharge	5	1	0.002	0.0045	0	0.01	0.01
	Propiconazole	Downstream	5	1	0.002	0.0045	0	0.01	0.01
	Propoxur	Influent	4	1	0.013	0.025	0	0.05	0.01
	Prosulfocarb	Upstream	5	1	0.002	0.0045	0	0.01	0.01
	Prosulfocarb	Discharge	5	1	0.002	0.0045	0	0.01	0.01
	Prosulfocarb	Downstream	5	1	0.002	0.0045	0	0.01	0.01
	Prosulfocarb	Further downstream	5	1	0.002	0.0045	0	0.01	0.01
	Prothioconazole	Upstream	5	1	0.004	0.0089	0	0.02	0.01
	Prothioconazole	Influent	4	2	0.13	0.15	0	0.3	0.01
	Prothioconazole	Effluent	4	2	0.05	0.06	0	0.12	0.01
	Prothioconazole	Discharge	5	2	0.014	0.019	0	0.04	0.01
	Prothioconazole	Downstream	5	2	0.01	0.014	0	0.03	0.01
	Prothioconazole	Further downstream	5	2	0.006	0.0089	0	0.02	0.01
	Simazine	Upstream	5	2	0.006	0.0089	0	0.02	0.01
	Simazine	Influent	4	2	0.0075	0.0096	0	0.02	0.01
	Simazine	Effluent	4	1	0.0025	0.005	0	0.01	0.01
	Simazine	Discharge	5	2	0.008	0.011	0	0.02	0.01
	Simazine	Downstream	5	2	0.006	0.0089	0	0.02	0.01
	Simazine	Further downstream	5	2	0.006	0.0089	0	0.02	0.01
	Spirotetramat-enol	Influent	4	3	0.018	0.013	0	0.03	0.01
	Spirotetramat-enol	Effluent	4	2	0.015	0.019	0	0.04	0.01
	Tebuconazole	Upstream	5	1	0.002	0.0045	0	0.01	0.01
	Tebuconazole	Influent	4	3	0.018	0.017	0	0.04	0.01
	Tebuconazole	Effluent	4	3	0.013	0.0096	0	0.02	0.01
	Thiabendazole	Effluent	4	1	0.0025	0.005	0	0.01	0.01
PFAS	5:3 FTCA	Influent	4	4	0.012	0.021	0.0004	0.043	0.0002
	6:2 FTAB	Upstream	5	1	0.0016	0.0036	0	0.008	0.0002
	6:2 FTAB	Discharge	5	1	0.00044	0.00098	0	0.0022	0.0002
	6:2 FTAB	Downstream	5	1	0.0005	0.0011	0	0.0025	0.0002

6:2 FTAB	Further downstream	5	1	0.00012	0.00027	0	0.0006	0.0002
6:2 FTS	Influent	4	4	0.0019	0.0020	0.0002	0.0045	0.0002
6:2 FTS	Effluent	4	3	0.00073	0.0012	0	0.0025	0.0002
6:2 FTS	Discharge	5	1	0.00004	0.000089	0	0.0002	0.0002
Linear PFHxS	Upstream	5	3	0.0086	0.018	0	0.04	0.0002
Linear PFHxS	Influent	4	4	0.086	0.16	0.0011	0.33	0.0002
Linear PFHxS	Effluent	4	4	0.11	0.21	0.0004	0.43	0.0002
Linear PFHxS	Discharge	5	3	0.0068	0.011	0	0.027	0.0002
Linear PFHxS	Downstream	5	3	0.0057	0.0088	0	0.021	0.0002
Linear PFHxS	Further downstream	5	3	0.0046	0.0076	0	0.018	0.0002
Linear PFOS	Upstream	5	3	0.0033	0.0055	0	0.013	0.0002
Linear PFOS	Influent	4	4	0.062	0.12	0.001	0.24	0.0002
Linear PFOS	Effluent	4	4	0.042	0.079	0.0002	0.16	0.0002
Linear PFOS	Discharge	5	3	0.0071	0.013	0	0.031	0.0002
Linear PFOS	Downstream	5	3	0.013	0.027	0	0.062	0.0002
Linear PFOS	Further downstream	5	3	0.0055	0.010	0	0.024	0.0002
PFECHS	Influent	4	1	0.00023	0.00045	0	0.0009	0.0005
PFECHS	Effluent	4	1	0.00023	0.00045	0	0.0009	0.0005
PFBS	Upstream	5	3	0.0019	0.0037	0	0.0086	0.0002
PFBS	Influent	4	4	0.014	0.023	0.0006	0.048	0.0002
PFBS	Effluent	4	4	0.018	0.034	0.0004	0.069	0.0002
PFBS	Discharge	5	3	0.0019	0.0026	0	0.0063	0.0002
PFBS	Downstream	5	3	0.0015	0.0019	0	0.0045	0.0002
PFBS	Further downstream	5	3	0.00094	0.0012	0	0.0026	0.0002
PFDA	Upstream	5	2	0.00024	0.00039	0	0.0009	0.0002
PFDA	Influent	4	2	0.0005	0.00058	0	0.001	0.0002
PFDA	Effluent	4	2	0.00053	0.00062	0	0.0012	0.0002
PFDA	Discharge	5	3	0.0003	0.00035	0	0.0008	0.0002
PFDA	Downstream	5	2	0.0004	0.00069	0	0.0016	0.0002

PFDA	Further downstream	5	2	0.0002	0.00027	0	0.0005	0.0002
PFHpS	Influent	4	2	0.0033	0.0065	0	0.013	0.0002
PFHpS	Effluent	4	2	0.0036	0.0070	0	0.014	0.0002
PFHpA	Upstream	5	3	0.00074	0.0014	0	0.0033	0.0002
PFHpA	Influent	4	4	0.0030	0.0047	0.0002	0.01	0.0002
PFHpA	Effluent	4	4	0.0042	0.0066	0.0003	0.014	0.0002
PFHpA	Discharge	5	3	0.0012	0.0016	0	0.0039	0.0002
PFHpA	Downstream	5	3	0.0009	0.0011	0	0.0026	0.0002
PFHpA	Further downstream	5	3	0.0007	0.00088	0	0.0021	0.0002
PFHxA	Upstream	5	3	0.0070	0.014	0	0.032	0.0002
PFHxA	Influent	4	4	0.027	0.046	0.0007	0.095	0.0002
PFHxA	Effluent	4	4	0.042	0.066	0.0048	0.14	0.0002
PFHxA	Discharge	5	3	0.0066	0.0082	0	0.02	0.0002
PFHxA	Downstream	5	3	0.0049	0.0057	0	0.014	0.0002
PFHxA	Further downstream	5	3	0.0040	0.0050	0	0.012	0.0002
PFNA	Upstream	5	2	0.0002	0.00035	0	0.0008	0.0002
PFNA	Influent	4	2	0.0011	0.0019	0	0.004	0.0002
PFNA	Effluent	4	2	0.0014	0.0024	0	0.005	0.0002
PFNA	Discharge	5	2	0.00026	0.00040	0	0.0009	0.0002
PFNA	Downstream	5	2	0.00036	0.00061	0	0.0014	0.0002
PFNA	Further downstream	5	2	0.00024	0.00036	0	0.0008	0.0002
PFOA	Upstream	5	3	0.0018	0.0033	0	0.0076	0.0002
PFOA	Influent	4	4	0.0078	0.013	0.0004	0.027	0.0002
PFOA	Effluent	4	4	0.011	0.015	0.0016	0.033	0.0002
PFOA	Discharge	5	3	0.0024	0.0025	0	0.0061	0.0002
PFOA	Downstream	5	3	0.0025	0.0027	0	0.0066	0.0002
PFOA	Further downstream	5	3	0.0018	0.002	0	0.0046	0.0002
PFPeS	Upstream	5	2	0.0011	0.0023	0	0.0051	0.0002
PFPeS	Influent	4	2	0.012	0.023	0	0.046	0.0002

	PFPeS	Effluent	4	2	0.015	0.030	0	0.061	0.0002
	PFPeS	Discharge	5	2	0.00082	0.0015	0	0.0035	0.0002
	PFPeS	Downstream	5	2	0.00048	0.00078	0	0.0018	0.0002
	PFPeS	Further downstream	5	2	0.00042	0.00069	0	0.0016	0.0002
	PFPeA	Upstream	5	3	0.0054	0.0099	0	0.023	0.0005
	PFPeA	Influent	4	4	0.017	0.020	0.0006	0.044	0.0005
	PFPeA	Effluent	4	4	0.032	0.030	0.0034	0.073	0.0005
	PFPeA	Discharge	5	3	0.012	0.015	0	0.033	0.0005
	PFPeA	Downstream	5	3	0.0080	0.0085	0	0.019	0.0005
	PFPeA	Further downstream	5	3	0.0057	0.0059	0	0.014	0.0005
	PFPrS	Influent	4	1	0.0025	0.005	0	0.01	0.0002
	PFPrS	Effluent	4	1	0.004	0.008	0	0.016	0.0002
	Total PFHxS	Upstream	5	3	0.010	0.021	0	0.048	0.0002
	Total PFHxS	Influent	4	4	0.094	0.17	0.0017	0.35	0.0002
	Total PFHxS	Effluent	4	4	0.11	0.22	0.0006	0.44	0.0002
	Total PFHxS	Discharge	5	3	0.0080	0.014	0	0.032	0.0002
	Total PFHxS	Downstream	5	3	0.0067	0.010	0	0.025	0.0002
	Total PFHxS	Further downstream	5	3	0.0056	0.0093	0	0.022	0.0002
	Total PFOS	Upstream	5	3	0.0053	0.0089	0	0.021	0.0002
	Total PFOS	Influent	4	4	0.11	0.20	0.0013	0.41	0.0002
	Total PFOS	Effluent	4	4	0.078	0.15	0.0004	0.3	0.0002
	Total PFOS	Discharge	5	3	0.0082	0.014	0	0.033	0.0002
	Total PFOS	Downstream	5	3	0.021	0.043	0	0.097	0.0002
	Total PFOS	Further downstream	5	3	0.0090	0.017	0	0.039	0.0002
Phthalates	Di-(ethylhexyl) phthalate	Upstream	5	2	0.048	0.10	0	0.23	0.01
	Di-(ethylhexyl) phthalate	Influent	4	4	2.08	1.32	0.9	3.8	0.01
	Di-(ethylhexyl) phthalate	Effluent	4	2	1.90	3.80	0	7.6	0.01
	Di-(ethylhexyl) phthalate	Discharge	5	2	0.042	0.078	0	0.18	0.01
	Di-(ethylhexyl) phthalate	Downstream	5	3	0.056	0.10	0	0.24	0.01

	Di-(ethylhexyl) phthalate	Further downstream	5	3	0.05	0.066	0	0.16	0.01
	Di-isobutyl phthalate	Upstream	5	1	0.004	0.0089	0	0.02	0.01
	Di-isobutyl phthalate	Influent	4	3	0.22	0.30	0	0.65	0.01
	Di-isobutyl phthalate	Effluent	4	1	0.013	0.025	0	0.05	0.01
	Di-isobutyl phthalate	Discharge	5	1	0.004	0.0089	0	0.02	0.01
	Di-isobutyl phthalate	Downstream	5	1	0.006	0.013	0	0.03	0.01
	Di-isobutyl phthalate	Further downstream	5	1	0.006	0.013	0	0.03	0.01
	Di-n-butyl phthalate	Upstream	5	3	0.058	0.057	0	0.13	0.01
	Di-n-butyl phthalate	Influent	4	1	0.018	0.035	0	0.07	0.01
	Di-n-butyl phthalate	Discharge	5	1	0.016	0.036	0	0.08	0.01
	Di-n-butyl phthalate	Downstream	5	1	0.028	0.063	0	0.14	0.01
	Di-n-butyl phthalate	Further downstream	5	2	0.046	0.078	0	0.18	0.01
	Di-n-pentyl phthalate	Influent	4	2	17.53	31.12	0	64	0.01
	Diethyl phthalate	Upstream	5	1	0.002	0.0045	0	0.01	0.01
	Diethyl phthalate	Influent	4	3	0.60	0.49	0	1.2	0.01
	Diethyl phthalate	Effluent	4	2	0.068	0.12	0	0.25	0.01
	Diethyl phthalate	Discharge	5	1	0.002	0.0045	0	0.01	0.01
	Diethyl phthalate	Downstream	5	1	0.002	0.0045	0	0.01	0.01
	Diethyl phthalate	Further downstream	5	1	0.002	0.0045	0	0.01	0.01
	Dimethyl phthalate	Influent	4	2	0.03	0.038	0	0.08	0.01
	Dimethyl phthalate	Effluent	4	1	0.005	0.01	0	0.02	0.01
Illicit PPCPs	Amphetamine	Influent	4	2	0.08	0.10	0	0.21	0.005
	Benzoylecgonine	Influent	4	4	0.57	0.21	0.31	0.77	0.005
	Benzoylecgonine	Effluent	4	4	0.11	0.16	0.024	0.35	0.005
	Benzoylecgonine	Discharge	5	1	0.0048	0.011	0	0.024	0.005
	Benzoylecgonine	Downstream	5	1	0.0058	0.013	0	0.029	0.005
	Benzoylecgonine	Further downstream	5	1	0.0016	0.0036	0	0.008	0.005
	Cocaine	Influent	4	3	0.12	0.11	0	0.24	0.005
	Cocaine	Effluent	4	1	0.016	0.032	0	0.063	0.005

	MDMA	Influent	4	4	0.037	0.020	0.008	0.055	0.005
	MDMA	Effluent	4	3	0.027	0.040	0	0.087	0.005
	Methamphetamine	Influent	4	4	1.13	0.68	0.2	1.8	0.005
	Methamphetamine	Effluent	4	3	0.13	0.16	0	0.35	0.005
	Methamphetamine	Discharge	5	1	0.0038	0.0085	0	0.019	0.005
	Methamphetamine	Downstream	5	2	0.004	0.0065	0	0.015	0.005
PPCPs	Acetaminophen	Influent	4	4	113.75	64.80	43	200	0.005
	Acetaminophen	Effluent	4	3	0.076	0.094	0	0.21	0.005
	Albuterol (Ventolin)	Influent	4	3	0.035	0.026	0	0.056	0.005
	Albuterol (Ventolin)	Effluent	4	3	0.0085	0.0070	0	0.017	0.005
	Amidotrizoate (sodium)	Influent	4	1	0.0098	0.020	0	0.039	0.005
	Amidotrizoate (sodium)	Effluent	4	1	0.005	0.01	0	0.02	0.005
	Amidotrizoate (sodium)	Discharge	5	2	0.0088	0.013	0	0.028	0.005
	Amidotrizoate (sodium)	Downstream	5	1	0.0048	0.011	0	0.024	0.005
	Amidotrizoate (sodium)	Further downstream	5	1	0.0052	0.012	0	0.026	0.005
	Asprin	Influent	4	3	0.024	0.030	0	0.064	0.005
	Atenolol	Influent	4	4	1.34	0.95	0.51	2.7	0.005
	Atenolol	Effluent	4	4	0.37	0.45	0.09	1.04	0.005
	Atenolol	Discharge	5	1	0.012	0.027	0	0.06	0.005
	Atenolol	Downstream	5	1	0.0094	0.021	0	0.047	0.005
	Atenolol	Further downstream	5	1	0.0016	0.0036	0	0.008	0.005
	Atorvastatin	Influent	4	3	0.73	0.56	0	1.3	0.005
	Atorvastatin	Effluent	4	4	0.097	0.11	0.012	0.26	0.005
	Atorvastatin	Discharge	5	1	0.0016	0.0036	0	0.008	0.005
	Azithromycin	Influent	4	3	0.19	0.14	0	0.34	0.005
	Azithromycin	Effluent	4	3	0.037	0.038	0	0.088	0.005
	Caffeine	Upstream	5	3	0.033	0.034	0	0.072	0.005
	Caffeine	Influent	4	3	50	45.28	0	100	0.005
	Caffeine	Effluent	4	3	0.49	0.65	0	1.4	0.005

Caffeine	Discharge	5	3	0.034	0.036	0	0.086	0.005
Caffeine	Downstream	5	3	0.032	0.041	0	0.1	0.005
Caffeine	Further downstream	5	3	0.023	0.030	0	0.073	0.005
Carbamazepine	Influent	4	4	0.52	0.16	0.36	0.71	0.005
Carbamazepine	Effluent	4	4	0.51	0.11	0.4	0.67	0.005
Carbamazepine	Discharge	5	2	0.042	0.069	0	0.16	0.005
Carbamazepine	Downstream	5	3	0.087	0.15	0	0.34	0.005
Carbamazepine	Further downstream	5	2	0.0096	0.014	0	0.032	0.005
Cefalexin	Influent	4	4	0.70	0.72	0.12	1.6	0.005
Cefalexin	Effluent	4	3	0.30	0.46	0	0.98	0.005
Cefalexin	Discharge	5	1	0.017	0.038	0	0.086	0.005
Cefalexin	Downstream	5	1	0.0024	0.0054	0	0.012	0.005
Cetrazine	Influent	4	4	0.50	0.36	0.042	0.88	0.005
Cetrazine	Effluent	4	4	0.28	0.21	0.13	0.59	0.005
Cetrazine	Discharge	5	2	0.015	0.027	0	0.062	0.005
Cetrazine	Downstream	5	2	0.029	0.044	0	0.1	0.005
Cetrazine	Further downstream	5	1	0.0016	0.0036	0	0.008	0.005
Citalopram	Influent	4	3	0.58	0.44	0	0.97	0.005
Citalopram	Effluent	4	4	0.083	0.10	0.01	0.23	0.005
Citalopram	Discharge	5	1	0.002	0.0045	0	0.01	0.005
Citalopram	Downstream	5	1	0.0036	0.0081	0	0.018	0.005
Clarithromycin	Influent	4	4	0.11	0.14	0.009	0.31	0.005
Clarithromycin	Effluent	4	2	0.02	0.034	0	0.07	0.005
Clindamycin	Influent	4	3	0.058	0.060	0	0.14	0.005
Clindamycin	Effluent	4	3	0.042	0.036	0	0.085	0.005
Clindamycin	Discharge	5	1	0.0028	0.0063	0	0.014	0.005
Clindamycin	Downstream	5	1	0.0058	0.013	0	0.029	0.005
Codeine	Influent	4	4	0.89	0.30	0.59	1.2	0.005
Codeine	Effluent	4	4	0.13	0.18	0.02	0.4	0.005

Codeine	Discharge	5	1	0.003	0.0067	0	0.015	0.005
Codeine	Downstream	5	1	0.0026	0.0058	0	0.013	0.005
Cotinine	Upstream	5	1	0.0028	0.0063	0	0.014	0.005
Cotinine	Influent	4	4	1.83	0.45	1.2	2.2	0.005
Cotinine	Effluent	4	3	0.028	0.037	0	0.083	0.005
Cotinine	Downstream	5	1	0.001	0.0022	0	0.005	0.005
Cyclophosphamide	Effluent	4	1	0.0015	0.003	0	0.006	0.005
Diclofenac	Influent	4	3	1.17	1.14	0	2.7	0.005
Diclofenac	Effluent	4	4	0.39	0.20	0.25	0.68	0.005
Diclofenac	Discharge	5	1	0.044	0.098	0	0.22	0.005
Diclofenac	Downstream	5	1	0.038	0.085	0	0.19	0.005
Diclofenac	Further downstream	5	1	0.0028	0.0063	0	0.014	0.005
Dicloxacillin	Influent	4	1	0.021	0.043	0	0.085	0.005
Doxylamine	Influent	4	4	0.29	0.17	0.12	0.52	0.005
Doxylamine	Effluent	4	4	0.24	0.056	0.2	0.32	0.005
Doxylamine	Discharge	5	1	0.026	0.058	0	0.13	0.005
Doxylamine	Downstream	5	1	0.036	0.080	0	0.18	0.005
Erythomycin	Influent	4	3	0.079	0.11	0	0.23	0.005
Erythomycin	Effluent	4	2	0.028	0.048	0	0.1	0.005
Erythomycin	Discharge	5	1	0.0016	0.0036	0	0.008	0.005
Erythomycin	Downstream	5	1	0.0022	0.0049	0	0.011	0.005
Fluoxetine	Influent	4	3	0.15	0.14	0	0.34	0.005
Fluoxetine	Effluent	4	1	0.002	0.004	0	0.008	0.005
Flurosemide	Influent	4	3	1.2	0.86	0	2	0.005
Flurosemide	Effluent	4	4	0.67	0.76	0.23	1.8	0.005
Flurosemide	Discharge	5	1	0.034	0.076	0	0.17	0.005
Flurosemide	Downstream	5	1	0.019	0.043	0	0.096	0.005
Flurosemide	Further downstream	5	1	0.0056	0.013	0	0.028	0.005
Gabapentin	Influent	4	4	10.38	7.11	6.3	21	0.005

Gabapentin	Effluent	4	4	1.45	0.96	0.6	2.7	0.005
Gabapentin	Discharge	5	2	0.086	0.13	0	0.29	0.005
Gabapentin	Downstream	5	3	0.067	0.089	0	0.17	0.005
Gabapentin	Further downstream	5	2	0.037	0.054	0	0.12	0.005
Ibuprofen	Influent	4	4	8.44	6.84	0.17	15	0.005
Ibuprofen	Effluent	4	1	0.073	0.15	0	0.29	0.005
Indometacin	Influent	4	3	0.085	0.071	0	0.17	0.005
Indometacin	Effluent	4	4	0.074	0.092	0.014	0.21	0.005
Indometacin	Discharge	5	1	0.0038	0.0085	0	0.019	0.005
Indometacin	Downstream	5	1	0.0026	0.0058	0	0.013	0.005
Ketoprofen	Influent	4	3	0.049	0.042	0	0.1	0.005
Ketoprofen	Effluent	4	1	0.013	0.025	0	0.05	0.005
Lamotrigine	Influent	4	4	0.67	0.20	0.44	0.84	0.005
Lamotrigine	Effluent	4	4	1.36	1.11	0.57	3	0.005
Lamotrigine	Discharge	5	3	0.20	0.27	0	0.54	0.005
Lamotrigine	Downstream	5	3	0.35	0.47	0	0.99	0.005
Lamotrigine	Further downstream	5	3	0.074	0.11	0	0.26	0.005
Levamisole	Influent	4	1	0.0033	0.0065	0	0.013	0.005
Levamisole	Effluent	4	2	0.0063	0.0078	0	0.016	0.005
Metformin	Upstream	5	3	0.017	0.025	0	0.06	0.005
Metformin	Influent	4	4	18.98	16.22	1.9	41	0.005
Metformin	Effluent	4	4	3.43	5.74	0.01	12	0.005
Metformin	Discharge	5	2	0.14	0.21	0	0.48	0.005
Metformin	Downstream	5	3	0.049	0.071	0	0.17	0.005
Metformin	Further downstream	5	2	0.053	0.080	0	0.18	0.005
Metoprolol	Influent	4	4	0.9	0.63	0.4	1.8	0.005
Metoprolol	Effluent	4	4	0.32	0.22	0.11	0.62	0.005
Metoprolol	Discharge	5	2	0.016	0.028	0	0.065	0.005
Metoprolol	Downstream	5	2	0.030	0.052	0	0.12	0.005

Metoprolol	Further downstream	5	1	0.0014	0.0031	0	0.007	0.005
Metronidazole	Influent	4	2	0.032	0.053	0	0.11	0.005
Metronidazole	Effluent	4	4	0.041	0.031	0.021	0.088	0.005
Metronidazole	Discharge	5	1	0.0028	0.0063	0	0.014	0.005
Minocycline	Influent	4	1	0.003	0.006	0	0.012	0.005
Mirtazampine	Influent	4	4	0.16	0.14	0.015	0.29	0.005
Mirtazampine	Effluent	4	4	0.019	0.014	0.007	0.037	0.005
Morphine	Influent	4	4	0.3	0.081	0.19	0.37	0.005
Morphine	Effluent	4	2	0.025	0.044	0	0.09	0.005
N-Desmethyl Citalopram	Influent	4	3	0.33	0.28	0	0.64	0.005
N-Desmethyl Citalopram	Effluent	4	3	0.042	0.054	0	0.12	0.005
N-Desmethyl Citalopram	Discharge	5	1	0.0014	0.0031	0	0.007	0.005
N-Desmethyl Citalopram	Downstream	5	1	0.0032	0.0072	0	0.016	0.005
Naproxen	Influent	4	4	2.71	3.83	0.056	8.4	0.005
Naproxen	Effluent	4	4	0.17	0.17	0.005	0.32	0.005
Nicotine	Upstream	5	4	0.027	0.021	0	0.055	0.005
Nicotine	Influent	4	4	3.08	1.13	1.7	4.4	0.005
Nicotine	Effluent	4	3	0.062	0.041	0	0.087	0.005
Nicotine	Discharge	5	2	0.016	0.023	0	0.048	0.005
Nicotine	Downstream	5	3	0.018	0.019	0	0.045	0.005
Nicotine	Further downstream	5	2	0.015	0.021	0	0.04	0.005
Octinoxate	Influent	4	3	0.17	0.20	0	0.46	0.005
Octinoxate	Effluent	4	2	0.011	0.013	0	0.026	0.005
Octocrylene	Upstream	5	1	0.034	0.076	0	0.17	0.005
Octocrylene	Influent	4	3	6.85	9.71	0	21	0.005
Octocrylene	Effluent	4	4	0.11	0.11	0.035	0.27	0.005
Octocrylene	Discharge	5	1	0.046	0.10	0	0.23	0.005
Octocrylene	Downstream	5	1	0.054	0.12	0	0.27	0.005
Octocrylene	Further downstream	5	1	0.048	0.11	0	0.24	0.005

Oxazepam	Influent	4	4	0.39	0.27	0.076	0.73	0.005
Oxazepam	Effluent	4	4	0.20	0.097	0.11	0.3	0.005
Oxazepam	Discharge	5	2	0.023	0.042	0	0.096	0.005
Oxazepam	Downstream	5	2	0.041	0.069	0	0.16	0.005
Oxazepam	Further downstream	5	2	0.0036	0.0054	0	0.012	0.005
Oxybenzone	Influent	4	2	0.12	0.14	0	0.28	0.005
Oxybenzone	Effluent	4	2	0.0075	0.0088	0	0.017	0.005
Paraben ethyl	Influent	4	2	0.0038	0.0048	0	0.01	0.005
Paraben methyl	Influent	4	1	0.038	0.075	0	0.15	0.005
Paraben propyl	Influent	4	3	0.046	0.035	0	0.082	0.005
Paraxanthine	Influent	4	4	4.15	1.50	2.7	6.2	0.005
Paraxanthine	Effluent	4	3	0.11	0.10	0	0.21	0.005
Phenytoin	Influent	4	3	0.88	1.05	0	2.1	0.005
Phenytoin	Effluent	4	3	0.73	1.08	0	2.3	0.005
Phenytoin	Discharge	5	2	0.0054	0.0074	0	0.014	0.005
Phenytoin	Downstream	5	2	0.008	0.011	0	0.024	0.005
Phenytoin	Further downstream	5	1	0.0018	0.0040	0	0.009	0.005
Roxithromycin	Influent	4	4	0.16	0.11	0.008	0.27	0.005
Roxithromycin	Effluent	4	3	0.032	0.031	0	0.07	0.005
Sertraline	Influent	4	1	0.088	0.18	0	0.35	0.005
Sulfadiazine	Influent	4	4	0.26	0.18	0.12	0.53	0.005
Sulfadiazine	Effluent	4	4	0.087	0.084	0.024	0.21	0.005
Sulfadiazine	Discharge	5	1	0.0072	0.016	0	0.036	0.005
Sulfadiazine	Downstream	5	1	0.0072	0.016	0	0.036	0.005
Sulfadiazine	Further downstream	5	1	0.0014	0.0031	0	0.007	0.005
Sulfamethoxazole	Influent	4	4	2.04	1.78	0.42	4.4	0.005
Sulfamethoxazole	Effluent	4	4	0.33	0.26	0.16	0.72	0.005
Sulfamethoxazole	Discharge	5	2	0.044	0.083	0	0.19	0.005
Sulfamethoxazole	Downstream	5	2	0.031	0.052	0	0.12	0.005

Sulfamethoxazole	Further downstream	5	2	0.009	0.012	0	0.023	0.005
Sulfathiazole	Influent	4	4	2.06	1.06	1.04	3.4	0.005
Sulfathiazole	Effluent	4	4	0.33	0.28	0.14	0.74	0.005
Sulfathiazole	Discharge	5	1	0.038	0.085	0	0.19	0.005
Sulfathiazole	Downstream	5	1	0.024	0.054	0	0.12	0.005
Sulfathiazole	Further downstream	5	1	0.0052	0.012	0	0.026	0.005
Temazepam	Influent	4	4	0.28	0.19	0.05	0.52	0.005
Temazepam	Effluent	4	4	0.19	0.073	0.11	0.28	0.005
Temazepam	Discharge	5	2	0.019	0.027	0	0.057	0.005
Temazepam	Downstream	5	2	0.036	0.050	0	0.1	0.005
Temazepam	Further downstream	5	2	0.0068	0.012	0	0.028	0.005
Tetracycline	Influent	4	2	0.025	0.044	0	0.09	0.005
Theophylline	Influent	4	3	0.048	0.037	0	0.088	0.005
Thiabendazole	Influent	4	3	0.006	0.0045	0	0.011	0.005
Thiabendazole	Effluent	4	2	0.011	0.018	0	0.037	0.005
Tramadol	Influent	4	4	0.57	0.12	0.42	0.69	0.005
Tramadol	Effluent	4	4	0.33	0.13	0.21	0.48	0.005
Tramadol	Discharge	5	2	0.020	0.037	0	0.085	0.005
Tramadol	Downstream	5	2	0.032	0.061	0	0.14	0.005
Tramadol	Further downstream	5	1	0.002	0.0045	0	0.01	0.005
Trichlosan	Influent	4	2	0.023	0.037	0	0.078	0.005
Triclocarban	Influent	4	1	0.005	0.01	0	0.02	0.005
Trimethoprim	Influent	4	4	0.40	0.25	0.15	0.7	0.005
Trimethoprim	Effluent	4	4	0.11	0.15	0.01	0.33	0.005
Trimethoprim	Discharge	5	1	0.0054	0.012	0	0.027	0.005
Trimethoprim	Downstream	5	1	0.0072	0.016	0	0.036	0.005
Valsartan	Upstream	5	1	0.0056	0.013	0	0.028	0.005
Valsartan	Influent	4	4	7.54	5.12	0.24	12	0.005
Valsartan	Effluent	4	4	2.07	2.89	0.53	6.4	0.005

	Valsartan	Discharge	5	3	0.11	0.17	0	0.4	0.005
	Valsartan	Downstream	5	3	0.064	0.070	0	0.16	0.005
	Valsartan	Further downstream	5	3	0.040	0.040	0	0.09	0.005
	Venlafaxine	Influent	4	4	0.54	0.20	0.35	0.79	0.005
	Venlafaxine	Effluent	4	4	0.35	0.11	0.25	0.47	0.005
	Venlafaxine	Discharge	5	1	0.026	0.058	0	0.13	0.005
	Venlafaxine	Downstream	5	1	0.054	0.12	0	0.27	0.005
	Warfarin	Influent	4	1	0.0015	0.003	0	0.006	0.005

Table S2. Summary statistics for estimated percent reduction (%R) of emerging contaminants where available, grouped across all sites sampled for wastewater. SD = standard deviation; Min = minimum; Max = maximum.

Analyte group	Analyte	Mean (%R)	SD (%R)	Min (%R)	Max (%R)
Artificial Sweeteners	Acesulfame K	95.85	3.52	90.91	98.5
	Cyclamate	92.25	8.73	79.31	98.4
	Saccharin	90.05	12.71	71.15	98.74
	Sucralose	-18.68	40.05	-76.47	16
EDCs	Androstenedione	89.71	14.56	79.41	100
	Androsterone	100	0	100	100
	BHT	84.12	22.46	68.24	100
	Bisphenol A	76.49	13.50	56.36	84.29
	Estriol	100	0	100	100
	Estrone	5.83	54.19	-40	83.33
	Etiocholanolone	100	0	100	100
	Nonylphenol	28.60	46.59	-4.35	61.54
	Testosterone	100	0	100	100
	tert-octyl phenol	-80.91	117.72	-164.15	2.33
Pesticides	Azoxystrobin	100	-	100	100
	Benalaxyl	100	0	100	100
	Carbendazim	0	-	0	0
	Cyromazine	71.43	-	71.43	71.43
	Deet	90.03	7.82	80	98.64
	Desethyl Atrazine	75	-	75	75
	Diazinon	100	-	100	100
	Diflufenican	75	-	75	75
	Diuron	-29.17	73.76	-100	50
	Dmst	100	-	100	100
	Fenhexamid	66.67	-	66.67	66.67
	Flutriafol	0	-	0	0
	Imidacloprid	-6.25	58.22	-60	60
	MGK-264	100	0	100	100
	Metsulfuron-methyl	66.67	57.74	0	100
	Permethrin, (1R)-cis	100	0	100	100
	Permethrin, (1R)-trans	100	0	100	100
	Piperonyl butoxide	100	0	100	100
	Propham	100	-	100	100
	Propiconazole	40	52.92	0	100
	Propoxur	100	-	100	100
	Prothioconazole	56.67	23.57	40	73.33
	Simazine	50	70.71	0	100
	Spirotetramat-enol	22.22	69.39	-33.33	100
	Tebuconazole	0	86.60	-100	50
	Thiabendazole	-35.93	177.22	-236.36	100
PFAS	5:3 FTCA	100	0	100	100
	6:2 FTS	52.78	41.94	0	100
	Linear PFHxS	36.96	47.22	-30.30	75
	Linear PFOS	38.66	33.57	13.79	87.5
	PFECHS	0	-	0	0

	PFBS	-5.45	91.50	-116.67	76.47
	PFDA	-5	21.21	-20	10
	PFHpS	-3.85	5.44	-7.69	0
	PFHpA	-41.25	31.19	-75	0
	PFHxA	-207.88	254.06	-585.71	-47.37
	PFNA	-12.5	17.68	-25	0
	PFOA	-166.62	181.69	-425	-22.22
	PFPeS	-28.80	5.38	-32.61	-25
	PFPeA	-329.39	308.41	-700	-65.91
	PFPrS	-60	-	-60	-60
	Total PFHxS	47.01	49.89	-25.71	87.76
	Total PFOS	37.44	26.85	15.38	76.47
Phthalates	Di-(ethylhexyl) phthalate (DEHP)	49.79	99.86	-100	100
	Di-isobutyl phthalate (DIBP)	90.20	16.98	70.59	100
	Di-n-butyl phthalate (DBP)	100	-	100	100
	Di-n-pentyl phthalate (DnPP)	100	0	100	100
	Diethyl phthalate (DEP)	93.06	12.03	79.17	100
	Dimethyl phthalate (DMP)	87.5	17.68	75	100
Illicit PPCPs	Amphetamine	100	0	100	100
	Benzoylecgonine	77.98	34.01	27.08	96.88
	Cocaine	66.67	57.74	0	100
	MDMA	39.62	89.42	-93.33	100
	Methamphetamine	89.24	14.60	68.18	100
PPCPs	Acetaminophen	99.86	0.24	99.51	100
	Albuterol (Ventolin)	74.73	10.49	67.74	86.79
	Amidotrizoate (sodium)	48.72	-	48.72	48.72
	Asprin	100	0	100	100
	Atenolol	61.35	47.80	-8.33	92.59
	Atorvastatin	82.96	20.49	59.38	96.46
	Azithromycin	81.98	7.94	74.12	90
	Caffeine	98.01	2.950	94.62	99.93
	Carbamazepine	-3.44	24.13	-19.51	32.39
	Cefalexin	61.82	45.51	-1.03	100
	Cetrazine	-16.28	132.10	-209.52	76.14
	Citalopram	87.09	9.73	76.29	95.18
	Clarithromycin	91.07	11.01	77.42	100
	Clindamycin	19.64	17.59	5.36	39.29
	Codeine	81.98	27.79	40.30	96.61
	Cotinine	98.32	2.075	95.39	100
	Diclofenac	58.80	40.93	11.69	85.56
	Dicloxacillin	100	-	100	100
	Doxylamine	-1.63	47.08	-66.67	38.46
	Erythomycin	56.94	42.86	14.29	100
	Fluoxetine	99.22	1.36	97.65	100

Flurosemide	45.53	64.21	-28.57	84.67
Gabapentin	79.91	17.91	57.14	96.14
Ibuprofen	98.71	2.59	94.82	100
Indometacin	12.75	106.43	-110	79.41
Ketoprofen	83.33	28.87	50	100
Lamotrigine	-119.92	204.44	-426.32	-9.52
Levamisole	30.77	-	30.77	30.77
Metformin	79.95	33.87	29.41	99.47
Metoprolol	54.21	42.52	-8.77	84.44
Metronidazole	-187.27	371.55	-450	75.45
Minocycline	100	-	100	100
Mirtazampine	69.74	29.70	36.21	96.55
Morphine	92.87	11.63	75.68	100
N-Desmethyl Citalopram	88.88	6.84	81.25	94.47
Naproxen	-41.05	263.33	-435.71	99.94
Nicotine	97.73	1.95	95.29	100
Octinoxate	95.56	7.70	86.67	100
Octocrylene	97.18	2.84	93.91	98.92
Oxazepam	28.38	51.28	-44.74	64.38
Oxybenzone	96.58	4.84	93.16	100
Paraben ethyl	100	0	100	100
Paraben methyl	100	-	100	100
Paraben propyl	100	0	100	100
Paraxanthine	97.58	2.64	94	100
Phenytoin	-24.60	90.24	-121.42	57.14
Roxithromycin	85.17	14.88	65	100
Sertraline	100	-	100	100
Sulfadiazine	59.37	39.02	4.55	87.92
Sulfamethoxazole	68.24	32.92	24.21	94.77
Sulfathiazole	76.04	31.49	28.85	93.24
Temazepam	-10.06	82.26	-120	69.23
Tetracycline	100	0	100	100
Theophylline	100	0	100	100
Thiabendazole	-35.93	177.22	-236.36	100
Tramadol	40.11	28.13	4.76	65.63
Trichlosan	100	0	100	100
Triclocarban	100	-	100	100
Trimethoprim	58.70	64.45	-37.5	98.57
Valsartan	20.02	111.30	-141.67	93.58
Venlafaxine	27.59	35.57	-17.14	68.35
Warfarin	100	-	100	100

Accessibility

Contact us if you need this information in an accessible format such as large print or audio.
Please telephone **1300 372 842** or email contact@epa.vic.gov.au

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