# Quaternary ammonium compounds (QACs) in wastewater influent and effluent throughout the COVID-19 pandemic

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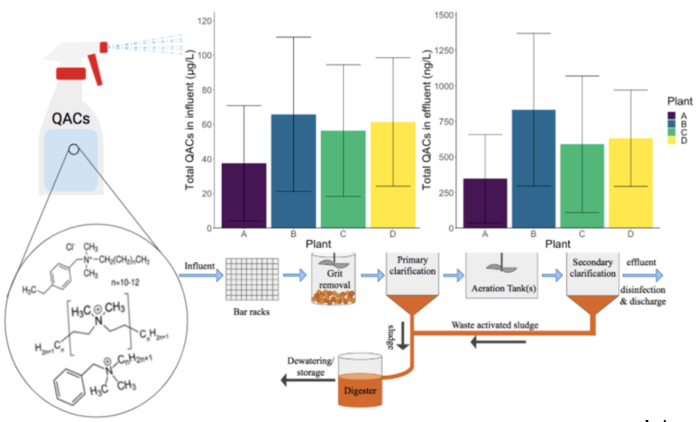


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**Abstract**

Quaternary ammonium compounds (QACs) are used in consumer and industrial products, including disinfectants. Due to the COVID-19 pandemic, disinfectant use has increased, purportedly increasing loads to wastewater treatment plants and the environment. To understand how the increased usage has affected QAC loadings to treatment plants and to determine how effectively plants remove QACs from liquid effluent that is discharged to surface and groundwaters, influent and effluent wastewater samples were collected from four treatment plants (treatment capacities <5 MGD to >100 MGD) for 21 months beginning in May 2020. Influent QAC concentrations were hundreds of μg/L and effluent QAC concentrations were $\leq$1 μg/L, corresponding to an average removal of 98% from all four plants. The most prevalent QACs in influent are those used most commonly in disinfectants, specifically benzylalkyldimethylammonium compounds (BACs) and short-chain dialkyldimethylammonium compounds (DADMACs), and influent levels of these compounds were correlated with QAC sales. Prior to this study, ethylbenzylalkyldimethylammonium compounds (EtBACs) had not been studied, and it was found they comprised 13 ± 6 % of QACs in influent. While removal was high at all plants, low μg/L concentrations are still continuously discharged into the environment. For QACs with equivalent alkyl chain lengths, those with an aromatic substituents (BACs and EtBACs) appear to be removed more effectively than those with only alkyl chains.

**Keywords**: wastewater treatment, COVID-19, disinfectants, benzylalkyldimethylammonium compounds (BACs), dialkyldimethylammonium compounds (DADMACs)

**Synopsis**: Quaternary ammonium compound (QAC) levels in wastewater influents are proportional to sales information but not to COVID cases, while effluent data shows effective, but not complete, removal of QACs.

## Introduction

Quaternary ammonium compounds (QACs), first used in the 1930s as surfactants and disinfectants, are used in many consumer, agricultural, healthcare and industrial products including fabric softeners, personal care products, disinfectants, biocides, sanitizers, and emulsifiers.[1–4] QACs are a class of chemicals that have a positively charged nitrogen atom with two methyl groups, a long alkyl chain of varying length (i.e., a homologous series) and a fourth side chain that varies in structure (Figure 1). DADMACs have two long alkyl chains which can have either the same or different chain lengths. These chemicals are designated as high production volume chemicals, with over one million pounds manufactured or imported annually.[5–7] Estimated reported production values show that up to 50 million pounds of certain

QACs are produced per year dating back to 2012.[8] Due to the COVID-19 pandemic, use of QAC containing products has increased, a trend that is expected to continue.[4,7,9,10] Currently, over 50% of the disinfectants on EPA list N: *Disinfectants for Use against SARS-CoV-2* contain QACs as an active ingredient.[7,11] Of those products, most contain benzylalkyldimethylammonium compounds (BACs) and/or

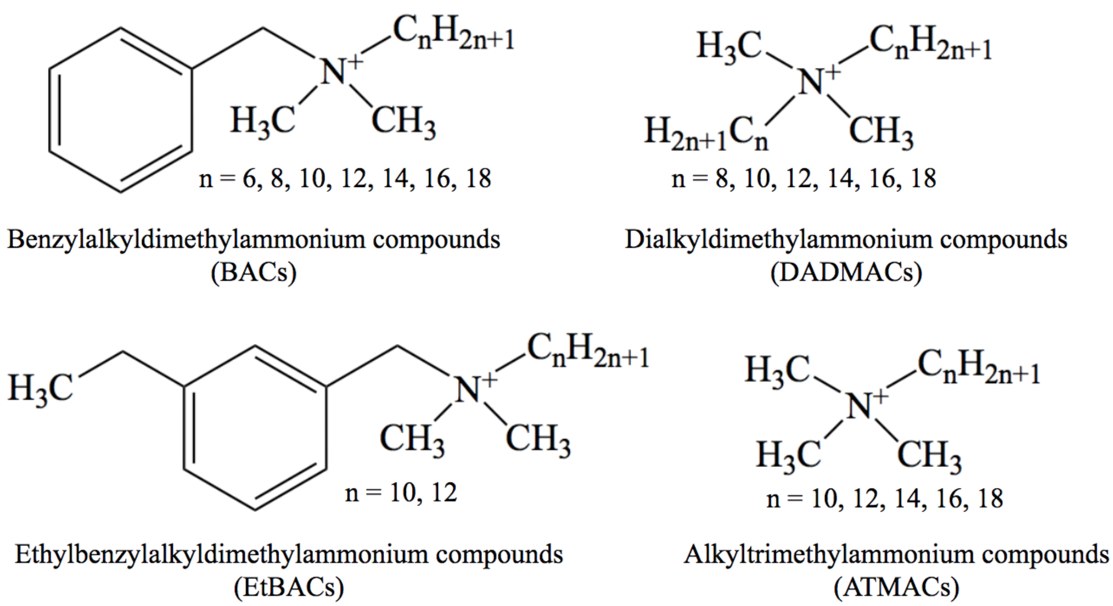


**Figure 1.** Generalized structures of the 4 classes of QACs. Each class of QAC contains a series of compounds with varying alkyl chain length.

dialkyldimethylammonium compounds (DADMACs) (93.5% and 49.5%, respectively). .[7,11] Ethylbenzylalkylammonium compounds (EtBACs) are present in 34% of the disinfectants, yet this is the first study to measure this class of QACs in wastewater. Chain lengths of QACs used in disinfectants include $C_8$- to $C_{18}$-BAC, $C_{12}$- and $C_{14}$-EtBACs and $C_8$-, $C_{8/10}$-, and $C_{10}$-DADMAC. Alkyltrimethylammonium compounds (ATMACs), along with other QACs such as

DADMACs (named DTDMAC in previous studies)[2,12] are used in fabric softeners and hair products.

Previous studies have shown that removal varies between compounds due to their different structures and chain lengths, which affects the hydrophobicity and solubility of each QAC.[13] Removal method can also vary based on structure; a more hydrophobic compound might sorb to the solids and be removed by sorption rather than biodegradation. Therefore, it is important to assess each of these QACs individually as well as cumulatively to obtain a full picture of the QAC profile in wastewater influents and effluents. Additionally, QACs have varying impacts on aquatic toxicity and microbial antibiotic resistance depending on structure, hence why many disinfectants contain multiple QACs.[2,14]

QACs are both water soluble and strongly sorb to solids and surfaces.[15,16] Thus, they have been previously detected in wastewater influent and effluent, surface waters, sediments and biosolids, and household dust.[15,17–2223] [23]Approximately 75% of QACs used worldwide annually end up in wastewater treatment systems, where 90% or greater are removed from the liquid phase mainly during activated sludge treatment.[1,16,24,25] Because QACs are highly sorptive, the main removal mechanism in wastewater treatment is sorption to activated sludge, but aerobic degradation also occurs.[16,25] While a large percentage of QACs are removed during treatment, these compounds are produced and used in such high volumes that the amount being released to the environment is still significant.[20] In addition, QACs can be discharged to the environment through land application of biosolids.

In disinfectant products, QACs are present at high levels to inactivate viruses and kill bacteria. In the environment, however, they are typically present at much lower, sub-lethal concentrations, implying that bacteria will not be killed, but could be stressed and adapt to the

QACs. Indeed, research has shown that QACs may contribute to the global antibiotic resistance problem.[14,26–29] A 2022 study investigated the induction of antibiotic resistance by $C_8$- to $C_{18}$-BACs and $C_{10}$-DADMAC on *E coli*. [14] The rate of adaptation of bacteria was faster for BACs, as DADMACs are more effective than BACs at inactivating bacteria and viruses.[14] More gene mutations and higher resistance levels developed, however, with exposure to DADMACs over an extended period of time.[14]

Before the COVID-19 pandemic, total QAC concentrations detected in effluent wastewater and surface water ranged from <1 μg/L to 4.1 μg/L, with one sample in Austrian hospital effluent containing 60 μg/L.[2,12,20–22,30–32] A 2019 Minnesota, USA study investigated 10 wastewater plants and detected effluent concentrations ranging from 0.4 to 8.3 μg/L, with higher levels of BACs and DADMACs than ATMACs.[31] Reported influent concentrations are up to an order of magnitude higher than the effluent concentrations, ranging from 1.4 to 170 μg/L, and similarly to effluents, concentrations of BACs and DADMACs were higher than ATMACs.[22,30,31,33,34] No study to date has investigated EtBACs in wastewater despite their widespread use in disinfectant products.

It is important to investigate how the increased use of QAC containing products during the COVID-19 pandemic has impacted their levels in wastewater and their removal during treatment. QACs have been shown to potentially disrupt and impact both the nitrification and denitrification processes in wastewater treatment plants.[35,36] A study found that while QACs entering plants at continuous and low concentrations did not have a significant impact on nitrification, high influx concentrations of 2 mg/L or more could cause serious issues, inhibiting carbonaceous reactions in aerobic systems for up to 48 hours.[35] Another study exclusively examined the effect of $C_{16}$-ATMAC on partial nitrification/anammox systems at a range of concentrations. Experimental levels were chosen to be 0.5 mg/L and 10 mg/L (both are higher than previously detected concentrations), and it was found that both levels could inhibit the anammox (*hzsB*) gene, thus impacting nitrogen removal.[35]

Many factors may impact removal of QACs during wastewater treatment. Operating temperature and the amount of dissolved oxygen (DO) present during activated sludge treatment could impact QAC degradation. Previous studies have shown that wastewater temperatures need to be above 12 °C with DO concentrations of at least 2 mg/L or higher for nitrification to efficiently occur in activated sludge systems.[37] In addition, size of treatment plant, the amount of QACs in influent, and additional treatment methods, such membrane filtration systems, could potentially impact removal of QACs.

This study was undertaken to determine the temporal loading and removal of of $C_6$-$C_{18}$-BACs, $C_8$-$C_{18}$-DADMACs, $C_{12}$-$C_{14}$-EtBACs and $C_{10}$-$C_{18}$-ATMACs in wastewater during the COVID-19 pandemic. Trends in the homologs present and concentrations over the course of the pandemic were also evaluated. No study to date has collected monthly QAC influent and effluent samples over a period of more than a year or analyzed a broad range of these compounds over time. QAC influent and effluent levels of four plants with a range of treatment capacities and treatment trains (see Table S1) were monitored from May 2020 to January 2022 to quantify QAC inputs and removal. Disinfectant sales data for the state of Minnesota and the formulations of QACs used in disinfectants on EPA List N were compared to the QAC concentrations in influents to assess the impact of disinfectant usage on the wastewater load. Trends during the pandemic for both total QAC concentrations ($[QAC]_{Total}$, the sum of target BACs, EtBACs, DADMACs, and ATMACs), or a subset of these compounds used in disinfectants,

$[QAC]_{disinfectants}$, and individual compounds were evaluated to assess any potential drivers that affected QAC use and presence in wastewater.

## Materials and Methods

A list of all chemicals used is given in the Supporting Information (SI). All abbreviations of QACs are listed in Table S2, as are vendors for all QAC standards, internal standards and surrogate standards. There were seven BACs, five ATMACs, two EtBACs and seven DADMACs that were monitored. The alkyl chain length of each compound is denoted by $C_x$ with x representing the number of carbons. For example, the compound dioctyldimethylammonium chloride is denoted as $C_8$-DADMAC. Ultrapure water (18.2 MΩ•cm at 25 °C) from a Milli-Q® system was used for sample blanks and water used in solid phase extraction. Tetraalkylammonium (TAA) compounds were used as internal standards (ISTDs).

*Wastewater sample collection*

Composite (24 hour) wastewater effluent and influent samples were collected monthly in 1-L polycarbonate bottles from May 2020 to May 2021 from four wastewater treatment plants (WWTPs) in the state of Minnesota, USA. Bottles were soaked in Alconox® (which is QAC-free) overnight, rinsed with deionized water then soaked in an acid bath (1 M HCl) overnight. After rinsing with ultrapure water, they were autoclaved for 30 minutes prior to sample collection. Samples were collected by plant personnel as part of their routine water quality analyses. After this period, quarterly samples from plants A, B and C were collected in July 2021, October 2021, and January 2022, while monthly samples from Plant D continued to be collected. The details of the WWTPs, including size and treatment methods, are listed in Table

S1. The approximate volume of each sample collected was 1 L, and due to limitations on laboratory activities at the initiation of the project due to the COVID-19 pandemic, the samples were frozen at -20 °C in the collection bottles. All samples, no matter when collected, were frozen for consistency. When extraction commenced, the samples were defrosted overnight and extracted the following morning. This allowed the samples to reach room temperature and large particulate matter to settle.

*Wastewater QAC extraction method*

A method was adopted that combined the water extraction method of Pati and Arnold[31] and the dust extraction method of Zheng,[23,31] because there was particulate matter in the samples even after settling which clogged solid phase extraction (SPE) cartridges. For each sample, two sub-samples were processed (one spiked with QACs to assess recovery and one unspiked). Each sample (250 g for effluent, 50 g for influent) was weighed into a combusted glass flask. Spiked effluent samples were amended with 200 µL of a 500 µg/L QAC stock solution of all target compounds in acetonitrile (ACN). All effluent samples (spiked and unspiked) were amended with 350 ng/L $C_{10}$-ATMAC-$d_9$ and $C_{14}$-BAC-$d_7$ as surrogates to test extraction efficiency. The spiked influent samples were amended with 400 µL of the 500 µg/L QAC solution, and 700 ng/L of the surrogates was added to all influent samples.

A ceramic Buchner funnel with combusted (500 °C, 4 hours) glass fiber filters (47 mm, Pall corporation) were used to filter the samples. After filtering, the filters were placed in a centrifuge tube, amended with 4 mL ACN, sonicated for an hour at room temperature and then centrifuged for 5 min at 1107 RCF.[23] After this, the ACN was carefully decanted into a combusted glass centrifuge tube, and the extraction steps were performed twice more for a total filter extraction volume of 12 mL ACN.

SPE of the filtered water was performed with 6 mL Oasis WCX cartridges (150 mg, 30 µm, Waters Corporation). Cartridges were first conditioned with 5 mL methanol (MeOH) and 5 mL ultrapure water. The filtered water was then loaded at a rate of approximately 5 mL/min. After the samples had been fully loaded, cartridges were washed with 5 mL ultrapure water and 5 mL MeOH, and then samples were eluted with 9 mL of ACN with 2% formic acid. The combined filter and SPE extracts were then evaporated to dryness under $N_2$ gas in a water bath at 40 °C. The extracts were reconstituted in 500 µL of ACN and filtered through a 0.2 µm PTFE filter (to protect the HPLC from any precipitates formed during the solvent evaporation that did not re-dissolve), before adding 450 µL of ultrapure water to the sample vials, and 50 µL of a 2000 µg/L internal standard (ISTD) solution was added for a total volume of 1 mL. Extracts were frozen at -20 °C until analysis.

Large particles settled out prior to filtration, but the filters captured some suspended particles, so the final reported concentrations represent "total dissolved QAC", defined as the sum of dissolved QACs, any QACs associated with dissolved organic matter, and QACs bound to small suspended, particles captured by the filter. Effluent samples from March and April 2021 were processed without the filter extraction step, because the SPE clogging issue was not noticed prior to extracting these two samples.

*Sample and data analysis*

Samples were analyzed by liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS), and the details, including a chromatogram showing all analytes, are in the SI.

Most QACs were target compounds for which authentic standards were used, but non-target analytes included $C_6$- and $C_8$- BAC, $C_{16}$-ATMAC and $C_{14}$-DADMAC. To calculate the concentrations of these analytes, the calibration curve of QAC that was most similar in structure and instrumental retention time was used to calculate the concentrations of the non-targets ($C_{10}$-BAC was used to calculate [$C_{6\text{-}8}$-BAC], $C_{14}$-ATMAC for [$C_{16}$-ATMAC] and $C_{12}$-DADMAC for [$C_{14}$-DADMAC]).[27] To correct for QAC losses during extraction, all analyzed concentrations were corrected for the relative recovery of the respective spiked sample, and these calculations are provided in the SI.[27] Due to low recoveries, some concentrations of long chain DADMACs, as well as $C_{18}$-ATMAC and $C_{18}$-BAC were corrected with a different process (see SI) that provides a conservative concentration value.

*Data Sources and Analysis*

The formulations of QAC-containing disinfectant products with respect to the ratio of BACs, EtBACs, and DADMACs were obtained from ref. [11] Daily influent and effluent temperatures and aeration basin DO were provided by all four plants over the sampling period. These variables were compared to concentrations and removals of QACs by averaging the daily temperature and DO values from the week prior to sample collection. Prior week averages were used (rather than single day data from the same date as sampling occurred) because same day data would likely not have impacted the composite wastewater samples that were analyzed. SARS-CoV-2 RNA (wastewater-based epidemiology) data from Plant D influent was obtained, starting in October 2020, and reported COVID-19 cases in Minnesota were compared to the QACs detected in influents using Pearson and Spearman correlations.[38] To compare daily COVID-19 cases to influent QAC levels, the average 7-day COVID-19 cases for the week prior to sample collection for Plants A-C, and average 7-day viral load for Plant D. In addition, averages of the month, two months, and 30-60 days prior were used, as consumer trends in disinfectant use might have a lag when compared to the COVID-19 curve. Viral RNA data may not match reported cases and are a more accurate measure of the amount of the SARS-CoV-2 virus in Plant D's sewershed.

Data were analyzed to assess if removal of QACs correlated to their individual differences in hydrophobicity. Octanol-water partition coefficients ($logK_{ow}$) of the QACs were obtained from or calculated using the methodology in Tezel.[39] Disinfectant sales data were acquired from the Minnesota Department of Agriculture's Pesticide Sales Database.[40–43] The 2020 and 2021 data for disinfectant QACs were compared to the average influent concentration of the same QACs over the respective years in wastewater influent from the same plants. All data were analyzed using the statistical tools in Microsoft Excel®, R[44], and R Studio. Spearman and Pearson correlations were calculated to assess relationships between QAC concentrations and removals to parameters including reported COVID-19 cases, SARS-CoV-2 RNA data, DO concentrations, influent temperatures and hydrophobicity. A Pearson test was used when the two parameters were expected to have direct proportionality and the measurements were on a numeric or interval scale (e.g., disinfectant sales vs. influent concentration). When the two parameters were not expected to be linearly correlated but could still show a relationship or one of the two parameters had low variation (e.g., DO), the rank order of the variables was determined and a Spearman test was performed. Correlation coefficients (r) with degrees of freedom and p-values are reported for all correlations ($\alpha$=0.05).

## Results

### *QACs in Wastewater Influent*

Detailed water quality, temperature, and COVID-19 data are in the SI, as are details for QAC extraction efficiency (average relative recoveries ranged from 20 to 140%). The influent $[QAC]_{Total}$ concentrations ranged from 4 to 174 μg/L (Figure 2). The reported concentrations in influents and effluents are the sum of the QACs from the extracted filter (non-settled solids) and dissolved in water. BACs were the group present in the highest concentrations in influent samples from Plants B, followed by DADMACs. Plant D had very similar concentrations of BACs and DADMACs, with slightly higher DADMAC concentrations on average. Plants A and C had a higher concentration of DADMACs than BACs. EtBAC levels were similar across plants and ATMACs were the class of QACs present in the lowest concentrations in influents from all four plants.

Each plant showed variations in concentrations over time. Plant A influent samples (Figure 2A) ranged in $[QAC]_{Total}$ from 4.1 to 33.8 μg/L until June 2021 when $[QAC]_{Total}$ increased, ranging from 44.8 to 100.3 μg/L from June 2021 to January 2022. Influents from Plant B (Figure 2B) had $[QAC]_{Total}$ ranging from 18.6 μg/L to 71.4 μg/L with the exception of three samples; April 2021, July 2021 and October 2021 which had a $[QAC]_{Total}$ of 173.8, 137.8 and 88.0 μg/L, respectively. Plant C influent (Figure 2C) samples all had concentrations ranging from 26.9 μg/L to 80.9 μg/L, except for the September 2020 sample, which had a $[QAC]_{Total}$ of 168.8 μg/L. Plant D (Figure 2D) influent had fluctuating QAC concentrations, ranging from 12.8 μg/L to 129.8 μg/L. For Plant D, $[QAC]_{Total}$ was higher than 120 μg/L in three samples; December 2021, June 2021 and October 2021.

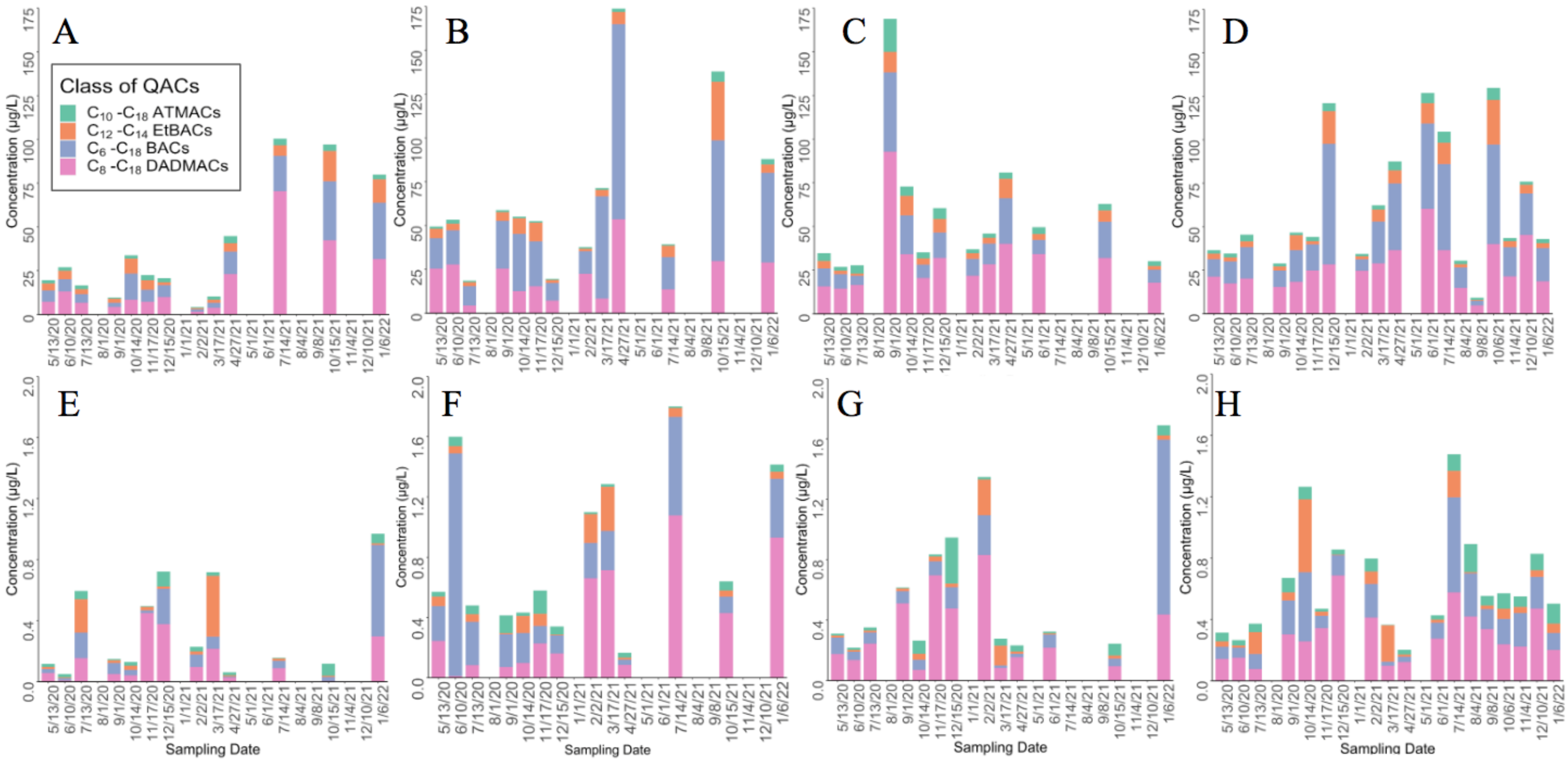


**Figure 2**. Panels A-D show concentrations in µg/L of the four classes of QACs in influent samples from Plants A, B, C and D from May 2020-January 2022, and panels E-H show the corresponding effluent measurements in µg/L. Note the change in y-axis scales. Dates where no data are shown indicate that no samples were collected that month. There were no samples in which no QACs were detected.

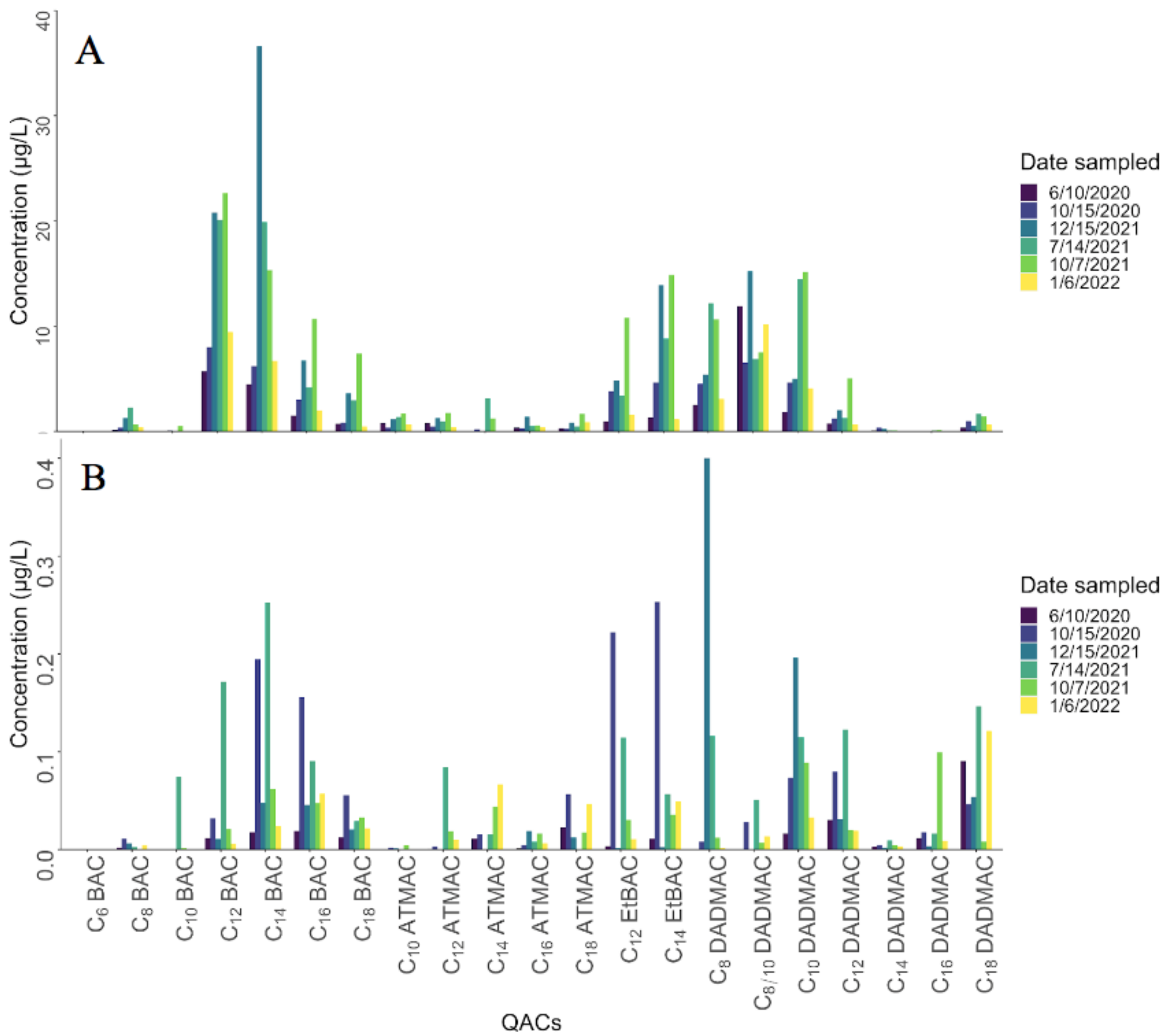


**Figure 3.** Individual QAC concentrations over time in influents (A) and effluents (B) from Plant D for 5 months between July 2020 and January 2021.

Individual QAC concentrations from Plant D across samples from six selected months (June, October and December 2020, July and October 2021 and January 2022), are shown in Figure 3A, and QACs present in the highest concentrations on average in the samples from each plant are listed in Table 1. There are high levels of $C_{12}$- to $C_{16}$-BACs, $C_8$- and $C_{10}$-DADMACs, and $C_{12}$- and $C_{14}$-EtBAC, which are all QACs used in disinfectants. Shorter chain BACs, longer chain DADMACs, and ATMACs are consistently lower in concentration than the QACs used in disinfectants. Longer chain QACs may have greater association with the settled solids, limiting their concentrations in the liquid samples. As shown in Table 1, all QACs present in the highest average concentration for all four plants were disinfectant QACs, with the exception of $C_8$-BAC in Plant B (see Table 1 footnote). $[QAC]_{disinfectant}$ refers to the total concentration of QACs used as active ingredients in disinfectants, $C_8$- to $C_{18}$-BAC, $C_{12}$- and $C_{14}$-EtBACs and $C_8$-, $C_{8/10}$-, and $C_{10}$-DADMAC. All QAC data for Plants A-D are in Tables S8-S15 and individual QAC concentration graphs for the same months are shown in Figures S5A–S7A.

**Table 1**. Individual QACs in influent (top) and effluent (bottom) displayed in order of the top eight QACs on average in Plant D. The bottom two QACs in the effluent table are those that were present in high concentrations on average in the other three plants. Reported errors are standard deviations (n=14 for Plants A-C, n=18 for Plant D). Note the different concentration units.

| Wastewater Influents | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Plant A | | Plant B[a] | | Plant C | | Plant D | |
| | Avg. (µg/L) | Max (µg/L) | Avg. (µg/L) | Max (µg/L) | Avg. (µg/L) | Max (µg/L) | Avg. (µg/L) | Max (µg/L) |
| $C_{8/10}$ DADMAC | 10 ± 14 | 49.2 | 8 ± 7 | 18.1 | 5 ± 3 | 12.5 | 13 ± 7 | 27.4 |
| $C_{14}$ BAC | 4 ± 5 | 16.2 | 5 ± 4 | 15.0 | 6 ± 4 | 15.2 | 10 ± 9 | 36.6 |
| $C_{12}$ BAC | 4 ± 4 | 11.0 | 5 ± 5 | 16.3 | 6 ± 6 | 23.0 | 10 ± 7 | 22.6 |
| $C_{10}$ DADMAC | 3 ± 3 | 11.7 | 9 ± 6 | 24.3 | 11 ± 9 | 37.5 | 6 ± 5 | 16.4 |
| $C_8$ DADMAC | 2 ± 1 | 3.8 | 2 ± 3 | 10.6 | 5 ± 3 | 12.5 | 6 ± 4 | 12.2 |
| $C_{14}$ EtBAC | 3 ± 3 | 12.4 | 4 ± 6 | 22.5 | 3 ± 2 | 6.6 | 4 ± 4 | 14.9 |
| $C_{16}$ BAC | 2 ± 2 | 5.3 | 2 ± 2 | 6.2 | 2 ± 1 | 4.9 | 3 ± 4 | 13.0 |
| $C_{12}$ EtBAC | 2 ± 2 | 5.7 | 3 ± 3 | 10.9 | 3 ± 2 | 6.3 | 3 ± 2 | 10.8 |
| Wastewater Effluents | | | | | | | | |
| | Avg. (ng/L) | Max (ng/L) | Avg. (ng/L) | Max (ng/L) | Avg. (ng/L) | Max (ng/L) | Avg. (ng/L) | Max (ng/L) |
| $C_8$ DADMAC | 30 ± 60 | 229 | 20 ± 20 | 82 | 30 ± 30 | 100 | 70 ± 110 | 400 |
| $C_{18}$ DADMAC | 60 ± 105 | 371 | 110 ± 220 | 705 | 120 ± 180 | 582 | 70 ± 70 | 209 |
| $C_{14}$ BAC | 30 ± 40 | 120 | 60 ± 70 | 225 | 50 ± 60 | 204 | 70 ± 60 | 253 |
| $C_{16}$ BAC | 10 ± 20 | 65 | 50 ± 40 | 160 | 20 ± 10 | 45 | 60 ± 40 | 156 |
| $C_{10}$ DADMAC | 20 ± 40 | 140 | 80 ± 120 | 399 | 70 ± 80 | 257 | 50 ± 50 | 196 |
| $C_{12}$ DADMAC | 10 ± 20 | 140 | 100 ± 120 | 419 | 40 ± 50 | 169 | 50 ± 40 | 122 |
| $C_{14}$ EtBAC | 30 ± 60 | 204 | 30 ± 20 | 71 | 60 ± 70 | 230 | 50 ± 60 | 253 |
| $C_{12}$ EtBAC | 30 ± 50 | 194 | 20 ± 20 | 88 | 20 ± 20 | 64 | 40 ± 60 | 222 |
| $C_{18}$ BAC | 3 ± 6 | 21 | 30 ± 60 | 217 | 12 ± 16 | 48 | 30 ± 20 | 62 |
| $C_{12}$ BAC | 50 ± 130 | 459 | 20 ± 20 | 88 | 88 ± 250 | 876 | 20 ± 40 | 171 |

[a]$C_8$ BAC was the highest concentration in Plant B influent (average=21 ± 23 µg/L, maximum of 80.3 µg/L). It was not present at this level in the other four plants so it is not included in this table.

Disinfectant sales data for the state of Minnesota showed that 300 metric tons of QACs ($C_8$–$C_{18}$-BACs, $C_{12}$-$C_{14}$-EtBACs, $C_8$, $C_{8/10}$, $C_{10}$ and $C_{18}$-DADMAC) were sold in 2021, of which 270 metric tons were sold specifically for disinfectants.[40,42,43] In 2020, 313 metric tons of these same QACs were sold, 280 metric tons of which were sold for disinfectant use.[40,42] This is higher than the amount of QACs sold for disinfectants the previous three years: 254 metric tons were sold in 2019, 266 metric tons in 2018, and 218 metric tons in 2017.[40–43] Of the 280 metric tons sold in 2020 as disinfectants, 25% was $C_{12}$-BAC, 18% was $C_{10}$-DADMAC and 17% was $C_{14}$-BAC.[40,42] These are the most common QACs used in disinfectant products, and they were the three most common QACs sold as disinfectants in 2021 as well.

Overall, 90% and 89% of all non-pesticide QACs sold in the state of Minnesota in 2021 and 2020, respectively, were for disinfectant use. There are other uses, such as fabric softeners and personal care products, that contain QACs. While data are not available for individual products, this database also had information on non-disinfectant QAC products used in households. In 2021, only 0.46 tons of QACs were sold for household purposes other than disinfectants, and thus most QACs sold for non-pesticide use in the state were for disinfectants.[40]

*QACs in Wastewater Effluents*

The $[QAC]_{Total}$ profile in effluents was slightly different than that in influents (Figure 2 panels E-H), with DADMACs again present in higher concentrations than BACs in effluents from all plants. BACs in effluents from Plant B were only slightly lower than DADMACs, and plants A, B and D all had higher concentrations of EtBACs than ATMACs, like in influents, while Plant C had higher concentrations of ATMACs.

All samples from Plant A (Figure 2E) had $[QAC]_{Total}$ less than 1 µg/L. The highest $[QAC]_{Total}$ detected was 0.97 µg/L in the January 2022 sample, and on average the $[QAC]_{Total}$ was at least half of that in the other plants' effluents. $[QAC]_{Total}$ in Plant B effluents (Figure 2F) varied substantially from month to month, with the three highest concentrations observed in June 2020, July 2021 and January 2022, with $[QAC]_{Total}$ of 1.6, 1.8 and 1.4 µg/L, respectively. While these samples had similar $[QAC]_{Total}$ concentrations, they were not comprised of the same classes; the June 2020 sample contained primarily BACs while the June 2021 and January 2022 samples contained primarily DADMACs along with higher levels of BACs and ATMACs. This plant had five months with $[QAC]_{Total}$ greater than 1 µg/L, more than any other plant. Most Plant C samples ranged in concentration from 0.22 – 0.95 µg/L, with two samples with a $[QAC]_{Total}$ greater than 1 µg/L: February 2021 (1.35 µg/L) and January 2021 (1.69 µg/L) (Figure 1G). Effluents from Plant D (Figure 2H) had the highest observed QAC concentrations in October 2020 and July 2021, with $[QAC]_{Total}$ equal to 1.27 µg/L and 1.48 µg/L, respectively.

Aside from the prevalence of long chain DADMACs in effluents, the QACs present in the highest concentrations in the effluent of Plant D were those present in influents and disinfectants (Figure 3B). Table 1 shows the highest concentration QACs in the effluent at all four plants. The individual QAC data from Plant D (Figure 3A & B) show the differences in trends between influent and effluent more closely. Graphs of individual QACs in effluents for the other three plants are in Figures S5B-7B. BACs and short chain DADMACs were clearly present in the highest amounts in influents, all fluctuating over time, while BACs were generally less prevalent and DADMACs were more prevalent in effluents for the same sampling months.

*Removal of QACs during treatment*

On average, all four plants removed at least 98% of the QACs present in influent from the liquid effluent, regardless of differences in plant size and treatment methods (Table 2). This does not mean that QACs were 98% removed from the total effluent, which includes biosolids. QACs have been shown to strongly sorb to solids, and while biosolids were not measured in the present study, some portion of the removed QACs likely adsorbed to biosolids. The samples collected were 24-hour composite samples. While the samples collected were not offset by the hydraulic retention time (i.e. they were collected at similar times), the composited samples should give a good approximation of removal. While pre-pandemic data is available from the same plants, only effluent data is available and only for one sampling date in November 2018, and those concentrations are similar to those measured in this study. While sales data and influent concentrations show increasing use, we cannot confidently state that levels in influent are higher than pre-pandemic levels. If levels have increased, there may be increased amounts of QACs present in biosolids, given the similar concentrations in effluents. This assumes that a large portion of QACs are removed through sorption to activated sludge, and therefore QAC levels in biosolids merit investigation.

**Table 2.** Summary of QAC influent, effluent, and removal for each wastewater treatment plant.

| Plant | Key features | Avg. influent $[QAC]_{Tot}$ (µg/L) | Avg. effluent $[QAC]_{Tot}$ (µg/L) | Avg. percent removal (%)[a] | Removal range (%) |
|---|---|---|---|---|---|
| Plant A | Membrane filtration & UV disinfection | 40 ± 30 | 0.4 ± 0.3 | 98 ± 2 | 93.1 - 99.9 |
| Plant B | UV disinfection | 60 ± 40 | 0.8 ± 0.5 | 98 ± 1 | 95.4 - 99.9 |
| Plant C | Aeration polishing pond & chlorination | 60 ± 40 | 0.6 ± 0.5 | 99 ± 2 | 99.7 – 94.4 |

| | | | | | |
|---|---|---|---|---|---|
| Plant D | Seasonal chlorination | 60 ± 40 | 0.6 ± 0.3 | 99 ± 1 | 94.0 - 99.8 |

[a][31]Average percent removal was determined by averaging each month's total QAC removal as recommended by the U.S. EPA.[45] Error limits are used to show the reproducibility, and as the ranges show, no removals were >100%.

Short chain ATMACs were regularly the class of QACs that were most successfully removed and DADMACs were least removed (Table S16). For example, $C_{10}$-ATMAC was 99 ± 1%, 95 ± 2%, 100 ± 0.1% and 100 ± 1% removed for Plants A-D, respectively. $C_{18}$-DADMAC had much lower removal in Plants A-D, with 89 ± 13%, 80 ± 30%, 92 ± 11%, 84 ± 15% removed, respectively. There were higher levels of ATMACs in effluents from Plant C than EtBACs, and there were very similar concentrations of the two classes in Plant D effluents. Effluents from Plants A and B contained more EtBACs than ATMACs. The individual QACs remaining varied from plant to plant, although all had substantial amounts of long chain DADMACs ($C_{14}$ – $C_{18}$) remaining. In all four plants' effluents the most prevalent class was DADMACs, followed by BACs.

## Discussion

*Comparison to previous measurements of QACs in wastewater influents and effluents*

Studies in Austria, China, Spain, Sweden and the United States have quantified QACs in influent and/or effluent (Table S17).[20–22,31,33,34,46–48] No studies were found that measured EtBACs. Influent individual QAC levels ranged from <LOD to 170 µg/L in other studies,[20,22,33,34,47,48] a similar range to that observed in the present study, in which influent individual QAC concentrations ranged from <LOD to 80.3 µg/L and total QACs from 0.43 to 313.4 µg/L. Martinez-Caraballo et al. measured BACs, DADMACs and ATMACs and found that BACs were present in the highest concentrations (0.068-170 µg/L), and ATMACs were

present in the lowest (0.049-0.95 μg/L), with the exception of one sample that had 9.1 μg/L of $C_{16}$-ATMAC.[20] $C_{12}$, $C_{14}$-BAC and $C_{12}$, $C_{16}$, $C_{18}$-DADMAC were all present, with highs of 170, 77, 7.2, 9.9 and 5.9 μg/L, respectively.[20] These results are consistent with the present study, implying QAC usage by chemical type is similar between Minnesota and Austria, and potentially other locations.

In effluents, the study by Martinez-Carballo et al. showed a similar trend to the effluents from Plants A-D; [20] similar concentrations of BACs and DADMACs were found (0.026 – 2.1 μg/L and 0.012 – 0.85 μg/L, respectively), and very little ATMACs were detected (n.d. – 0.40 μg/L).[20] Individual QAC concentrations in this study ranged from non-detect to 2.1 μg/L, and in all studies effluent values ranged from non-detect to 4.1 μg/L while total QACs ranged from 0.01 to 8.27 μg/L. [20,21,31,33,46–48] Again, these ranges are similar to the range of QACs detected in effluent from Plants A-D, in which concentrations ranged from <LOD to 1.80 μg/L. Pati et al. showed that, on average, $C_{18}$-DADMAC had the highest concentration in effluents (1.03 μg/L), followed by $C_{14}$ to $C_{18}$-BACs (0.22, 0.28, 0.11 μg/L, respectively), and $C_{10}$–$C_{14}$-DADMAC (0.20, 0.12, 0.12 μg/L, respectively). [31] ATMACs were detected in the lowest concentrations, all <0.01 μg/L.[31] These data are similar to the effluent data presented herein.

In dust particles collected in Indiana homes before and during the COVID-19 pandemic, the three QACs detected regularly in the highest concentrations of QACs measured ($C_{12}$, $C_{14}$-BAC and $C_{10}$-DADMAC) are the three QACs most commonly used in disinfectants (Table S18), which implies that disinfectants were the largest source of household QACs.[23] BACs were present in the highest concentrations in dust particles, a result that is also seen in Plant A, B, and D influents.[23] Neither this study, nor any others, investigated $C_{8/10}$-DADMAC, a common disinfectant QAC, and that is likely why the present study detected more DADMACs. Overall, the percent of QAC classes detected for all plants are similar to these dust particle results, which supports the interpretation that use of QACs as disinfectants is leading to their detection in wastewater influents.[23]

Of all QACs measured, $C_8$-BAC was present in the highest concentrations in Plant B influents and effluents, on average 21 ± 23 µg/L and 0.7 ± 0.4 µg/L respectively, but it was not observed in high concentrations in any of the other plants (averages ranged from ~0.16-0.75 µg/L in influents and 0.005–0.008 µg/L in effluents). This QAC is used much less frequently in products than the other BACs, EtBACs and DADMACs, and thus these are surprising results. It is present in disinfectants in the same proportion as $C_{10}$-BAC, which was not detected in high concentrations in any of the samples from Plant B. It is unlikely that homologues would degrade to $C_8$-BAC, as alkyl chains are known to degrade via oxygenases, and BACs also undergo degradation at the ring.[49] This presents a research gap on the importance of biodegradation pathways of QACs. One possible explanation is that there is an industrial source contributing a large amount of this QAC to the plant that is not present in the other sewersheds. There are a number of businesses in the Plant B sewershed (hospital, breweries, hair salons, funeral homes) that could be potential sources.

*Factors Affecting Influent QAC Concentrations*

There are five mixtures of BACs, one mixture of EtBACs and three mixtures of DADMACs commonly used in disinfectant products (Table S19-S20).[11] In three of the mixtures, $C_{14}$-BAC is present in the highest proportion, and in the other two $C_{12}$-BAC is highest. Spearman correlations were used to compare percentages in disinfectant mixtures to average percent of BACs in influents from each plant. When comparing the ranking of mixture 4 (Table S19) to the average BACs in influents, there were significant correlations found for all four plants (Table S22), with the same significance for Plants A and B ($\rho(4)=0.880$, $p=0.021$) and the same for Plants C and D ($\rho(4)=0.941$, $p=0.005$). There are also mixtures that contain BACs and DADMACs. Plotting the influent concentrations with these mix formations (Figure S8) demonstrates that the patterns are largely similar, with the key exception that influents are skewed towards $C_{12}$-BAC compared to the marketed mixtures. This provides additional evidence that disinfectant usage is driving the presence of QACs in wastewater. When all BAC mixtures were compared to percentages of each BAC in influent (for example, $[C_{12}\text{-BAC}]/[\text{BAC}]_{TOTAL}$) , statistically significant correlations were observed for all plants; Plants B-D had the same relationship ($\rho(4)=0.943$, $p=0.005$) and Plant A had the strongest possible correlation ($\rho(4)=1.000$, $p=<0.0001$). When all mixtures were averaged together for the percentages of BACs, EtBACs and DADMACs used in disinfectants (Table S22) and compared to the same QACs in influents, Plants A ($\rho(9)=0.791$, $p=0.004$), B ($\rho(9)=0.873$, $p=0.0005$) and C ($\rho(9)=0.818$, $r=0.002$) and D ($\rho(9)=0.782$, $p=0.004$) had significant correlations at the 95% CI.

The 2020 sales data for individual QACs was strongly correlated to the influent concentrations of individual $[\text{QAC}]_{\text{disinfectants}}$, with Plants C ($r(10)=0.789$, $p=0.002$) and D ($r(9)=0.792$, $p=0.002$) having the strongest Pearson correlations (Figure S9) Plants A ($r(10) = 0.582$, $p = 0.047$) and Plant B ($r(10) = 0.702$, $p = 0.016$) were significant at the 95% confidence interval as well. The four plants (Plant A ($r(10) = 0.783$, $p = 0.004$), Plant B ($r(9)=0.835$, $p=0.010$), Plant C ($r(10) = 0.609$, $p = 0.036$) and D ($r(10)=0.882$, $p=0.0002$)) all had significant correlations at the 95% confidence interval with the 2021 disinfectant sales data as well (Figure S9).

Information about all Pearson and Spearman correlations performed is presented in Tables S21-S22. One potential driver of QAC influent concentration is statewide reported

COVID-19 cases and/or SARS-CoV-2 RNA data. For Plant D, SARS-CoV-2 RNA data were available, which is more reliable than daily COVID-19 cases as it directly measures the amount of virus in the region served by the wastewater treatment plant, whereas statewide reported COVID-19 cases may be inaccurate due to lack of self-reporting or testing, especially as at-home testing became popular. There were no significant correlations calculated using the Pearson or Spearman methods between the reported COVID-19 cases (one month, two months, or 30-60 days prior) and monthly $[QAC]_{Total}$ in influent samples. Similarly, viral loads and monthly $[QAC]_{Total}$ were not correlated either. The top 5 highest concentration QACs (Table S22) for each plant were compared to the COVID-19 related data as well, and again, no correlations were found.

The lack of correlations with COVID-19 cases does not necessarily mean that the COVID-19 pandemic is not influencing disinfectant use, especially because the sales data indicates increasing use.[4,7,9,10] Disinfectant usage likely does not align perfectly with COVID-19 cases; more disinfection might have occurred in the first several months of the pandemic when disinfection of every surface was highly encouraged, which is not when cases were the highest in Minnesota. In addition, industrial and commercial applications of disinfectants likely regularly occur due to more rigid cleaning practices (e.g., in food production) and might not have fluctuated drastically throughout the pandemic. Lastly, another potential reason for the lack of correlations is that samples were only taken once a month at most and continuous data would be better for assessing these correlations. Nonetheless, there is consistent input of QACs to wastewater treatment plants.

*Factors affecting QAC removal*

The wastewater for this study was collected from four plants with differing sizes and treatment methods. Despite differences in treatment trains and sizes, 98% of QACs were removed from the liquid effluent in all plants, showing that, even in systems without specialized treatment (such as membrane filtration) or in large capacity systems (such as Plant D), QACs are being successfully removed from liquid. In comparing the average effluent values in Table 2 or the values in in Nov. 2020 (0.49, 0.57, 0.83, and 0.47 μg/L, for plants A-D respectively) to those measured in Nov. 2018 (0, 0.5, 0.1, and 0.5 μg/L), there is not a clear difference in what is being discharged to the environment, but it not possible to be certain given only one sample point prior to the COVID-19 pandemic from these plants.

QACs are removed in wastewater treatment by a combination of aerobic biodegradation and sorption to the activated sludge.[13,16,39] Certainly, a portion of the removal observed is merely phase transfer, and future evaluation of concentrations in biosolids is needed. Overall, all BACs, ATMACs and EtBACs were removed more successfully than long chain DADMACs. While no studies to date have investigated the degradation of EtBACs, a few studies have compared degradation of BACs and ATMACs with equivalent alkyl chain lengths. Overall, these studies found that biodegradability decreases with increasing alkyl chain length, and further decreases when a benzyl group is substituted in place of a methyl group.[2,16,50–53] In one study, the degradation rate of $C_{14}$-ATMAC was five times faster that of $C_{14}$-BAC.[53] Another study found that DADMACs, with two alkyl chains, degrade five times slower than ATMACs. Overall, ATMACs have been shown to aerobically degrade the fastest. Because EtBACs are structurally similar to BACs, it would be likely based on this information that their biodegradation would be slower than ATMACs too. [53] This potentially explains why long chain DADMACs appear to have very low removal compared to the other compounds; their degradation is much slower and thus the retention times of the plants may not be long enough for these compounds to degrade.

$K_{ow}$ values (Tables S23-24) were used to quantify whether hydrophobicity correlated to QAC removal during treatment. Correlations were used to determine 1) how hydrophobicity of the different classes affected removals and 2) whether chain length impacts removal within QAC classes. When log$K_{ow}$ values were compared to percent of QACs removed during treatment for 11 QACs in all four plants, significant, yet negative, Pearson and Spearman correlations resulted (Figure S9). For these correlations only removal data for $C_{12}$ and $C_{16}$-ATMAC, $C_{12}$–$C_{16}$-BACs and $C_8$–$C_{10}$, $C_{14}$–$C_{16}$ DADMACs were used. The strongest Pearson correlations occurred for the percent removed from Plants C ($r(10)=0.901$, $p=<0.001$) and D ($r(10)=0.927$, $p=<0.0001$) (Figure S9). QACs that are removed by sorption are removed from the liquid effluent, but are not removed from the total ouflows from the plant because any QACs sorbed to biosolids are leaving the treatment plant.

When looking specifically at removal based on chain length in specific classes, there were no observed Pearson or Spearman correlations for ATMACs from any plants. The increasing chain length of BACs was only correlated with percent removal from Plant B at the 90% CI ($r(5)=0.751$, $p=0.052$). Increasing chain length of DADMACs was negatively correlated with removal from all plants (Plant A: $r(5)=0.784$, $p=0.037$, Plant B: $r(5)=.885$, $p=0.008$, Plant C: $r(5)=0.921$, $p=0.003$, Plant D: $r(5)=0.892$, $p=0.007$), all at the 95% confidence interval.

The negative correlations observed contradict previous data from papers showing that the more hydrophobic a QAC is, the more easily it is removed by sorption to activated sludge.[13] A study by Ismail et al. investigated sorption of four QACs ($C_{12}$-ATMAC, $C_{16}$-ATMAC, $C_{12}$-BAC and $C_{16}$-BAC) to four different types of sludges and found that the longer the chain length, the higher the extent of sorption.[13] The type of sludge did not matter as much as the structure of the QACs. While this study did not investigate as many QACs and did not investigate any DADMACs, the results imply that higher chain length compounds should be better removed by sorption. This is not what our Pearson or Spearman correlation coefficients indicate for all groups except BACs in Plant A wastewater. One potential reason for this is that shorter chain QACs have been shown to be more biodegradable, leading to better removal than longer chain QACs. [53] The negative correlations all mean that as chain length increases, percent remaining in effluent increases, which potentially implies that as chain length increases, removal decreases at these plants. While $K_{ow}$ may predict sorption to sludge, these results show that it does not necessarily predict overall removal, which is a combination of biodegradation and sorption.

*Environmental Significance*

This study was the first to collect influent and effluent samples from a set of wastewater treatment plants over an extended time period, and the first to report EtBAC concentrations. EtBACs are ingredients in many widely used products and these results indicate that EtBACs should be evaluated when studying environmental QACs because they make up more than 10% of the QAC load. Toxicity and impacts on antibiotic resistance from environmental levels of EtBACs should be studied along with the more common BACs and DADMACs. Thus, it is important to study their presence and removal in wastewater. The QACs detected in the highest concentrations were almost all QACs used in disinfectants, and sales data correlated to levels in influent, indicating use of QACs as disinfectants are driving inputs to wastewater treatment plants. If sales continue to increase, it is likely loads to the plants will as well. While COVID-19 cases/SARS-CoV-2 RNA data were not a direct indicator of QAC concentrations, increased use/sales of QACs for disinfectants driven by the COVID-19 pandemic appears to be an important factor in loadings. While most QACs are being successfully removed (~98% for all plants) despite varying unit operations, the balance of sorption to biosolids and biodegradation needs further study. Over half of the biosolids in the United States are land applied, thus providing another potential pathway for QACs into the environment. Even with effective removal, levels in the range of 1-2 μg/L total QACs are still being released into the environment which is higher than many other classes of emerging contaminants present in wastewater effluent. Because QACs have been linked to antibiotic resistance, further study of QACs and associated resistance genes in surface waters receiving effluents is warranted.

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## Supporting Information

List of chemicals, detailed experimental procedures, analytical procedure, LC-MS/MS parameters, statistical correlations, water quality data, COVID-19 data and influent and effluent QAC concentrations for all compounds tested. This information is available at http://pubs.acs.org.

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