# Screening for Emerging Chemical Contaminants in Water Using LC/Q-TOF and Mass Profiler Professional Software

## Application Note

Environmental

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## Abstract

The Agilent 6540 Q-TOF LC/MS and Agilent Mass Profiler Professional Software (MPP) were used to sensitively characterize the complex chemical composition of municipal wastewater. The processing of Q-TOF high-resolution MS data using the MPP multivariate statistical analysis package revealed changes in the occurrence patterns of organic chemicals during water treatment. Using this technique, we were able to determine which organic contaminants were attenuated, resilient, and formed during ozonation of recycled water. Heat maps created in MPP provide patterns that can be used to assess subtle changes in water quality and to identify emerging contaminants unique to a particular water or treatment process.

## Agilent Technologies

## Introduction

A plethora of reports have shown trace levels of unregulated contaminants (aka emerging contaminants) in water supplies [1] and in drinking water [2]. Those chemicals detected represent a very small portion of the approximately 7,500 chemicals listed in the US Environmental Protection Agency (EPA) Contaminant Candidate List universe (http://water.epa.gov/scitech/drinkingwater/dws/ccl/ccl3.cfm) and an even smaller portion of the nearly 85 million chemicals assigned CAS numbers (https://www.cas.org/). Numerous chemicals exist in water that have not yet been identified, and largely have an unknown impact on environmental and public health.

Specifically, regional water resources are facing unprecedented stress due to water shortages resulting from rapid population growth and relocation [3]. Many metropolitan areas are seeking additional resources to augment drinking water supplies [3]. The most likely new resource is water reuse, involving recycling wastewater into drinking water. Considering the daunting number of chemicals identified and potentially present in municipal wastewater, monitoring programs struggle to select those indicator compounds that are most representative of treatment efficacy. Perhaps more difficult is the nearly unlimited number of transformation products that may form when organic contaminants are subjected to oxidative or biological treatment processes. In some cases, byproducts of treatment are more toxic than the initial contaminants [4]. A survey method is needed that can monitor all of these processes to ensure selection of the most efficient and cost-effective treatments for wastewater reuse.

Accurate-mass quadrupole time-of-flight (Q-TOF) spectrometry is an excellent platform for detecting and resolving trace levels of thousands of different organic compounds in water, making it ideal as a monitoring tool for wastewater treatment. This application note describes a method that has been developed to profile the organic contaminants in wastewater before and after ozonation, a technology commonly used for disinfection and organic contaminant attenuation. The method developed employs ultra high performance liquid chromatography (UHPLC) and accurate-mass spectrometry, using an Agilent 1290 Infinity LC System and an Agilent 6540 Q-TOF LC/MS to separate and detect thousands of organic compounds in water. Clusters of compounds with similar fate during a given treatment process were elucidated, and some of these compounds could be used as indicators of the efficiency of the oxidation process. Finally, should a contaminant become of particular interest in the future, the Q-TOF data will constitute an archive that could be mined again, providing historical data without the necessity of storing or reanalyzing any sample.

## Experimental

### Reagents and standards

All solvents used were of highest purity available and suitable for LC/MS analysis. Methyl tertiary butyl ether (MTBE), methanol, and HPLC grade water used for solid phase extraction were procured from Fisher Scientific. Acetonitrile and HPLC grade water used for chromatography were obtained from Burdick and Jackson, while the additive formic acid was procured from Sigma-Aldrich.

### Instruments

This study was conducted using an Agilent 1290 Infinity LC System coupled to an Agilent 6540 Ultra High Definition (UHD) Accurate-Mass Q-TOF LC/MS system. The instrument conditions are shown in Table 1.

Table 1.  LC and Q-TOF MS Conditions

**LC run conditions**

| | |
|---|---|
| Column | Agilent ZORBAX Eclipse Plus 50 × 2.1 mm, 1.8 µm (p/n 959757-902) |
| Column temperature | 35 °C |
| Injection volume | 3 µL (three replicate injections of each sample) |
| Mobile phase | A) 0.1% formic acid in water v/v |
| | B) 0.1% formic acid in acetonitrile v/v |

| Linear gradient | Time (min) | %A | %B |
|---|---|---|---|
| | 0 | 95% | 5% |
| | 1.5 | 95% | 5% |
| | 10 | 0% | 100% |
| | 13 | 0% | 100% |
| Flow rate | 0.4 mL/min | | |

**Q-TOF MS conditions**

| | |
|---|---|
| Ion mode | ESI positive, MS only |
| Nebulizer gas | 40 psi |
| Capillary voltage | 4,000 V |
| Acquisition mode | 2 GHz |
| Acquisition range | 25–3,200 $m/z$ |
| Acquisition speed | 2.5 spectra/sec |
| Resolving power | 20,000 at $m/z$ = 400 |
| Accuracy | < 2 ppm |

## Sample preparation

Samples were collected from a wastewater treatment plant located in Arizona, where some of the secondary wastewater effluent was partially diverted through an ozonation pilot. Samples were collected after treating the secondary wastewater with 0, 1.5, 3, 4.5, or 5.6 mg/L of ozone.

Each sample was then filtered through a glass fiber filter (Whatman GF/F). Solid phase extraction was performed using cartridges preconditioned with 5 mL of MTBE, 5 mL of methanol and 5 mL of reagent grade water. The water sample (375 mL) was loaded onto the cartridge, which was then rinsed with 5 mL of reagent grade water and dried for 30 minutes under a nitrogen stream. The cartridge was then sequentially eluted with 5 mL of methanol and 5 mL of methanol/MTBE (10/90). The eluate was evaporated down to 0.5 mL under a nitrogen stream.

## Data analysis

The data were processed using Molecular Feature Extractor (an algorithm detecting compounds based on isotopic ion clusters) in the Agilent MassHunter software suite, followed by compound alignment using the Agilent Mass Profiler Professional (MPP) multivariate statistical analysis package. To discard artifacts, the aligned features were then subjected to recursive analysis using the Find Compound by Formula

tool in MassHunter, followed by a second round of compound alignment in MPP. Statistical analysis to identify profile differences between sample types was also performed using MPP.

## Results and Discussion

### Feature extraction and compound alignment

While total ions chromatograms (TICs) of triplicate injections overlap, and some differences can be observed corresponding to the different levels of ozone treatment, they are too complex for direct interpretation (Figure 1). Feature extraction is necessary to extract compounds from each chromatogram and establish a detailed profile of each sample. However, between two injections, a compound might have an insignificant shift in retention time that would cause MassHunter to identify it as two different entities. To correct for this, after features were extracted in MassHunter they were subjected to compound alignment using MPP. This process identified a total of 24,779 compounds. Initial filtering by occurrence (blank subtraction) identified 23,574 compounds detected only in wastewater samples. Of these, 13,996 compounds were detected at least twice (either in different replicates or in different samples), and were used for recursive analysis to further eliminate any false positives.
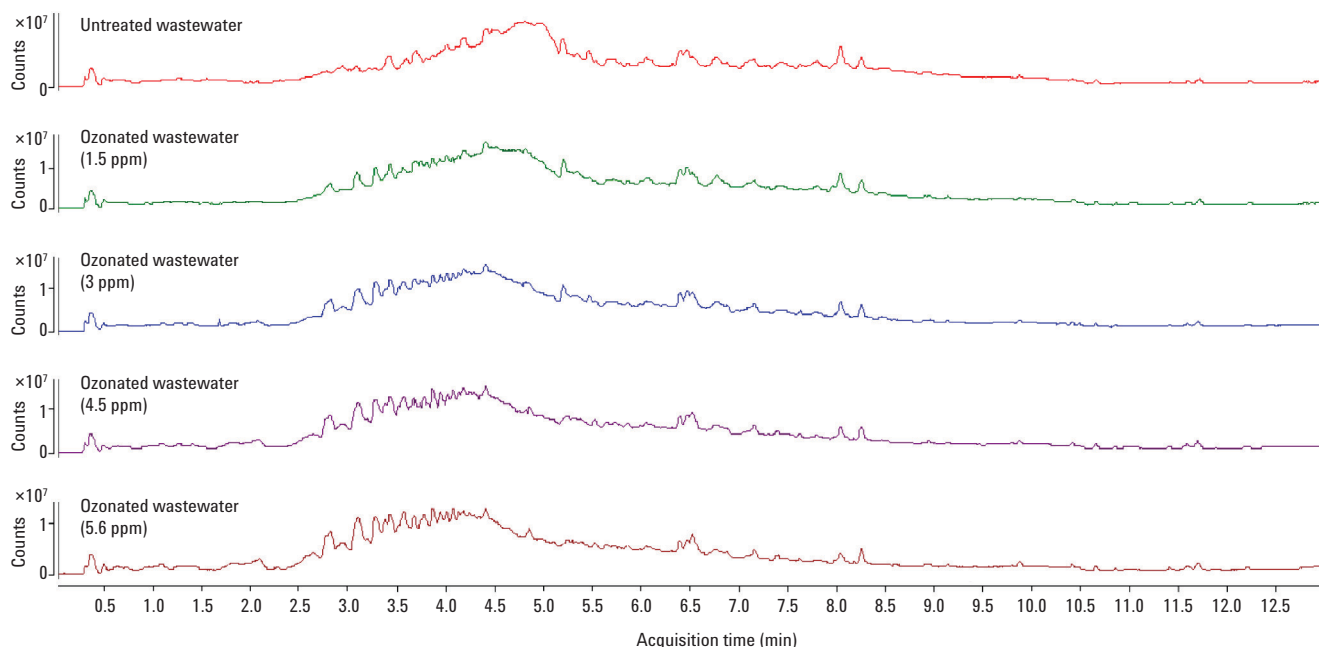


Figure 1.  Total ions chromatograms (TICs) for untreated wastewater and wastewater treated with various doses of ozone.

## Recursive analysis

The 13,996 compounds that passed the initial filtering were exported in a CEF file and imported into MassHunter Qualitative Analysis Software. The chromatograms were examined again to search particularly for these compounds using the Find Compound by Formula tool, and then the resulting compounds were imported back into MPP for a second round of compound alignment and filtering. A total of 12,889 compounds passed this recursive analysis. Of these, only those compounds that did not appear in blanks (extracted HPLC grade water) and were detected in 100% of the triplicate analyses for at least one of the ozone dose levels were selected for statistical analysis, leaving a total of 9,493 compounds.

## Principal component analysis

Principal Component Analysis (PCA) was performed on the data using MPP to determine if the samples could be distinguished based on the ozone dose that they received. Figure 2 shows that every ozone level could be distinguished from the others, and the triplicate samples taken at each level cluster very closely, indicating excellent reproducibility.

## Hierarchical clustering analysis

To further characterize and clearly observe the impact of ozonation on compounds present in wastewater, Hierarchical Clustering Analysis (HCA) was performed on the samples, revealing several groups of compounds that differed in their response to ozonation (Figure 3). Two groups, (A and B) were removed by ozone, while three others (C, D, and E) were formed by ozone treatment. Several compounds were actually resistant to ozone treatment (F). Additional detail can be observed in the HCA. For example, differences in the level of ozone required to remove compounds can be seen in Group B, revealing three subgroups (B1, B2, and B3, Figure 4).
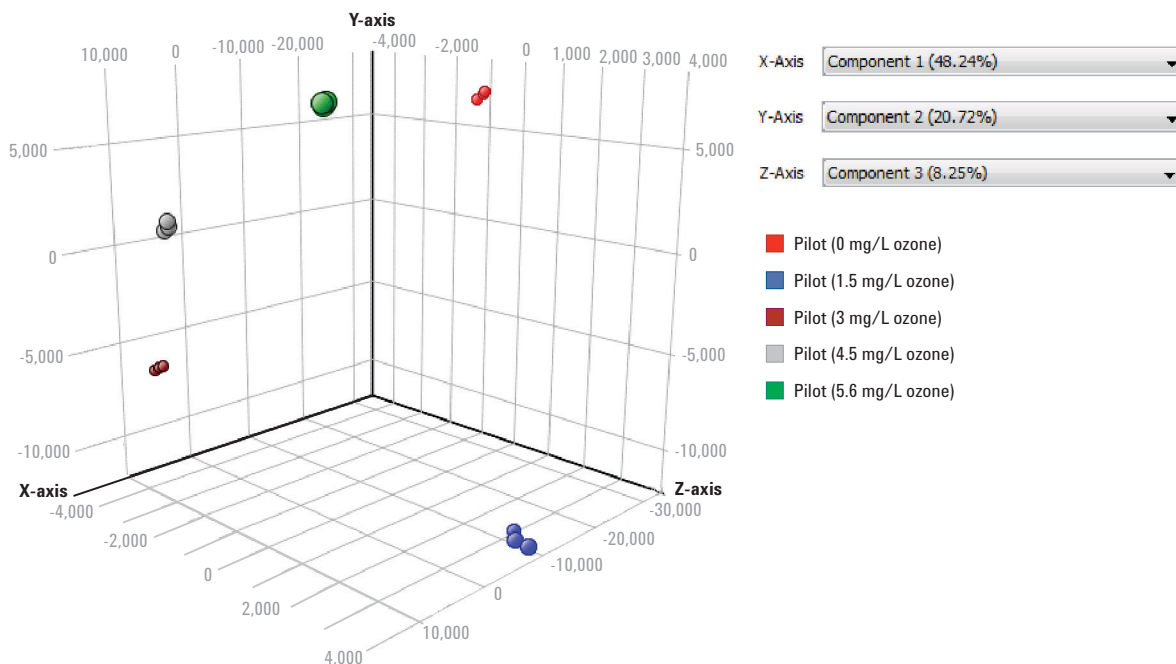


Figure 2. PCA reveals that samples treated with different doses of ozone can be clearly differentiated. The triplicates in each sample group also cluster very closely together.
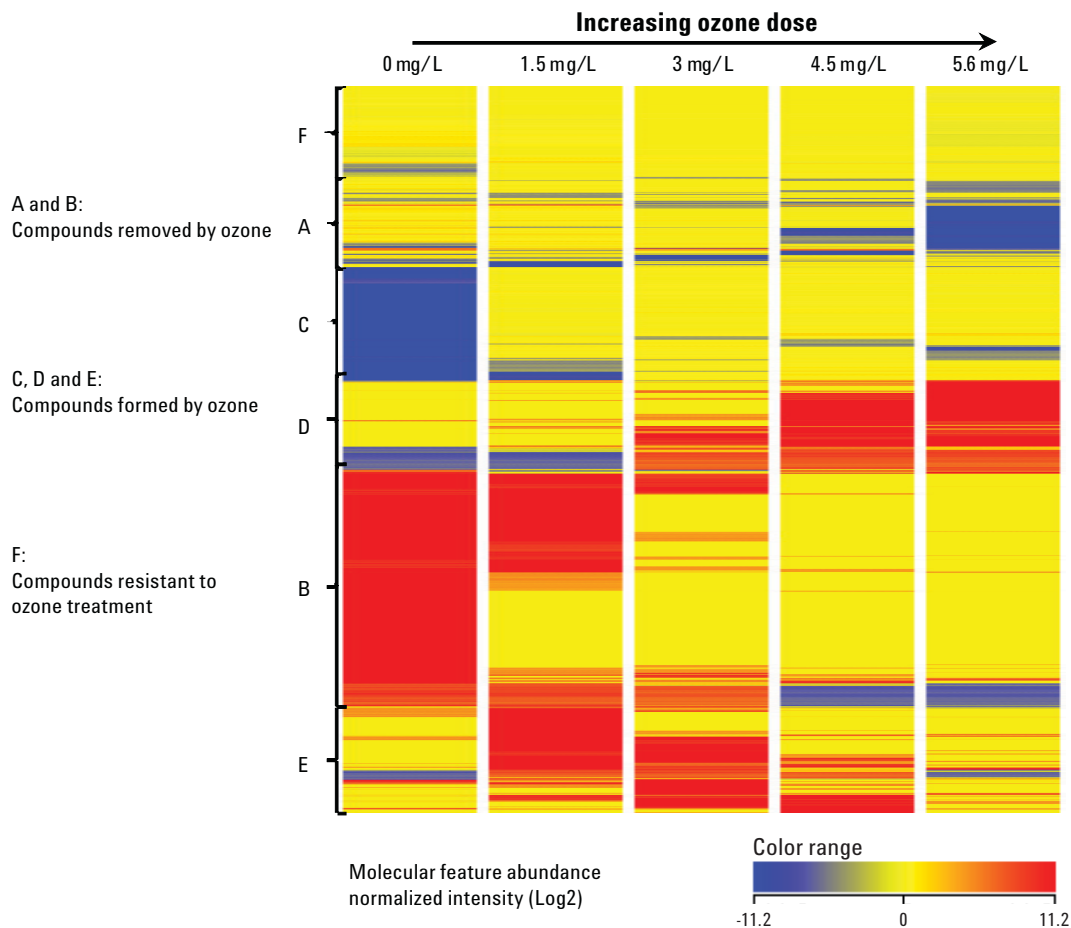
**Increasing ozone dose**

0 mg/L  1.5 mg/L  3 mg/L  4.5 mg/L  5.6 mg/L

A and B:
Compounds removed by ozone

C, D and E:
Compounds formed by ozone

F:
Compounds resistant to
ozone treatment

F

A

C

D

B

E

Molecular feature abundance
normalized intensity (Log2)

Color range

-11.2    0    11.2

Figure 3.  HCA, revealing groups of compounds that respond differently to ozone treatment.

**Increasing ozone dose**

0 mg/L  1.5 mg/L  3 mg/L  4.5 mg/L  5.6 mg/L

B1:  Compounds removed by
4.5 mg/L ozone or more

B2:  Compounds removed by
4.5 mg/L ozone or more
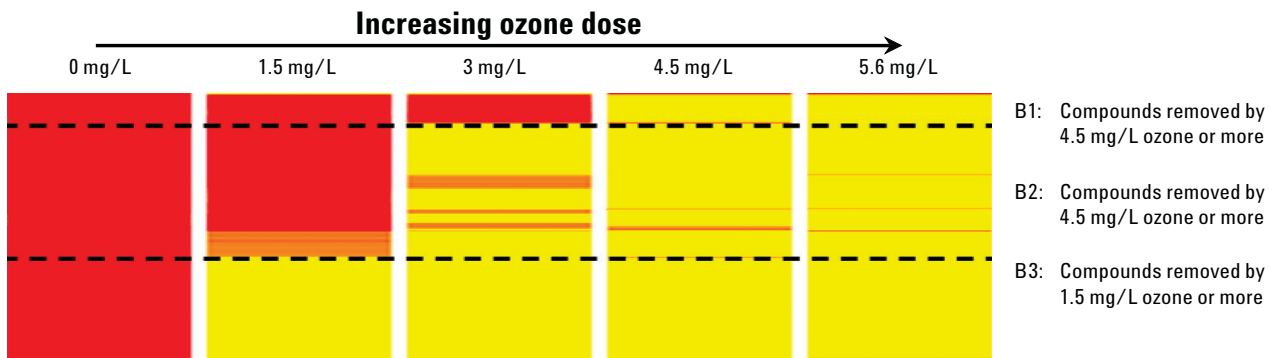
B3:  Compounds removed by
1.5 mg/L ozone or more

Figure 4.  Expanded view of Group B from Figure 3, showing the three subgroups (B1, B2, and B3) that differ in the level of ozone required to remove them from
the wastewater.

## Analysis of variance

To further understand the impact of various ozone treatments on wastewater, an analysis of variance (ANOVA) was performed in combination with Tukey's test to identify those compounds whose abundance varied significantly between the various ozone doses applied. The ANOVA identified 8,244 compounds out of 9,493 that had a significant variation of abundances (p value <0.05) dependent on the ozone dose. Tukey's test provided a table comparing each sample treated by a different ozone dose, indicating the number of compounds that did or did not significantly vary in abundance (Table 2).

## Identification of compounds

It is not feasible to monitor all compounds in water to assess their removal. Therefore, an alternative is identifying one compound in each cluster (B1, B2...) that could be used as an indicator to assess the fate of the other chemicals with similar behavior. This can be done using ID Browser in MPP and a database of suitable compounds. For example, when randomly selecting a compound in the subgroup B3, the Find Similar Entities tool in MPP identified 953 compounds that had a similar fate during ozonation with a correlation coefficient $0.95 < R^2 < 1$. This list was exported to ID Browser, which searched the Agilent METLIN Personal Compound Database and Library (PCDL) for matches. One of the compounds was identified as warfarin, the most widely prescribed anticoagulant drug in North America (Figure 5). Therefore, it could be used as an indicator for the fate of the 952 other compounds that are also easily removed by ozone (subgroup B3).

Table 2. *Number of Compounds That Vary in Abundance with Ozone Dose (ANOVA with p value < 0.05)*

| | | Ozone dose (ppm) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 1.5 | 3.0 | 4.5 | 5.6 |
| Ozone dose (ppm) | 0 | 8244 | 4548 | 5524 | 6083 | 6150 |
| | 1.5 | 3736 | 8244 | 3083 | 4299 | 4843 |
| | 3.0 | 2720 | 5161 | 8244 | 2476 | 3395 |
| | 4.5 | 2161 | 3945 | 5768 | 8244 | 2226 |
| | 5.6 | 2094 | 3401 | 4849 | 6018 | 8244 |

Compounds that vary in abundance
Compounds that do not vary in abundance
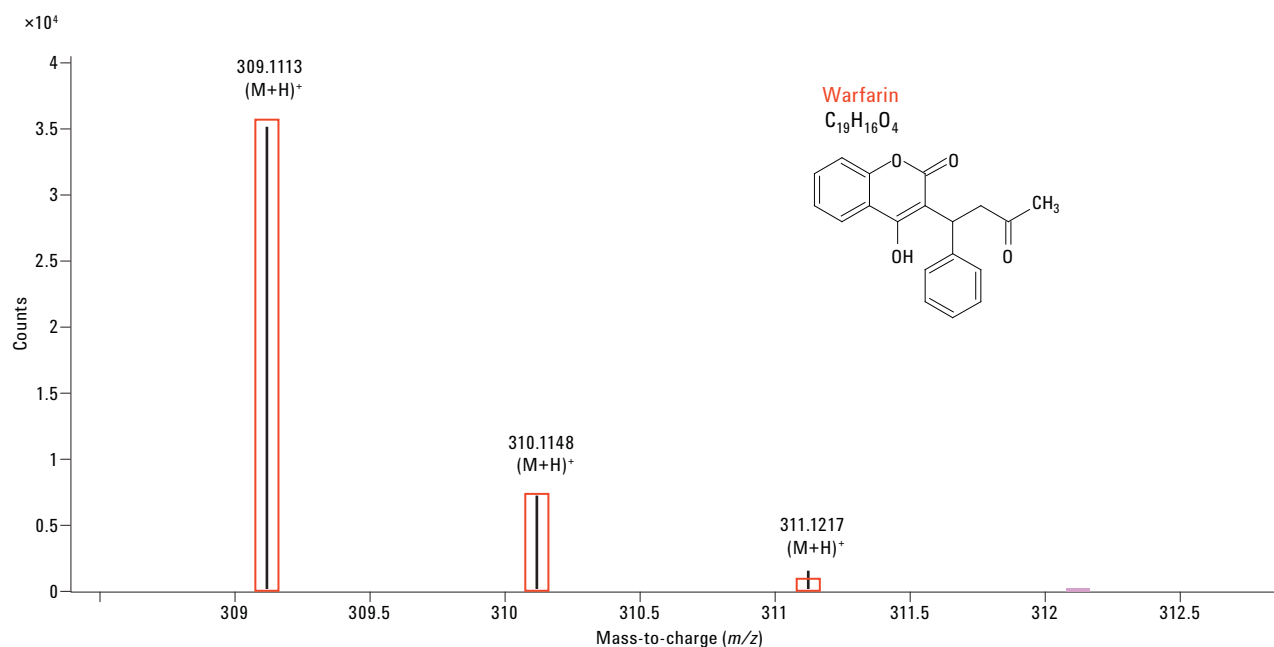Comparison of one sample group to itself



Figure 5. *One of the compounds in subgroup B3 identified as warfarin using MPP ID Browser and the Agilent METLIN PCDL database. The identification score was 97 (of a maximum of 100), and the red rectangles represent the theoretical ion cluster for warfarin.*

6

## Conclusions

Wastewater contains a very large number of compounds, most of which are not monitored in routine analysis. Using untargeted LC/Q-TOF analysis on the Agilent 6540 Ultra High Definition (UHD) Accurate-Mass Q-TOF LC/MS system and statistical analysis with Agilent Mass Profiler Professional software can differentiate groups of compounds with different vulnerability to ozone. Some compounds are resilient to ozonation, others are more or less easily attenuated depending on the ozone dose and, finally, some are formed by ozonation. Therefore, the method presented in this application note can be used as a sensitive monitoring tool for the changes in water quality that occur during water treatment.

## References

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