Traffic, drugs, mental health, and disinfectants: changes in sewage sludge chemical signatures during a COVID-19 community lockdown

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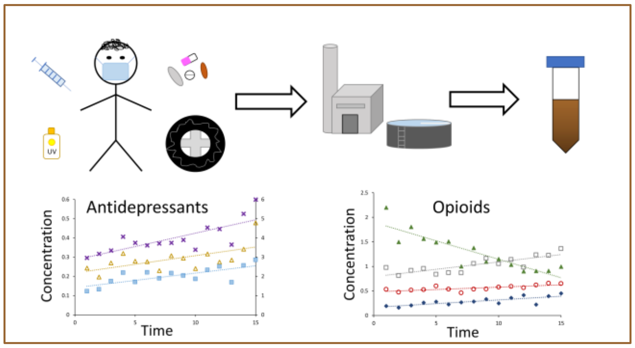
Abstract: The COVID-19 pandemic has caused lifestyle changes for many people, and signs of those changes are present in sewage sludge. We analyzed primary sludge from a wastewater treatment plant in Connecticut, USA collected March 19 to June 30, 2020. This time period encompassed the first wave of the pandemic, initial statewide stay at home order, and first phase of reopening. We used liquid chromatography high resolution mass spectrometry and targeted and suspect screening strategies to identify contaminants. We found evidence of increasing opioid, cocaine, and antidepressant use, and upward trends in chemicals used in disinfectants and sunscreens. Benzotriazole, an anti-corrosion chemical associated with traffic pollution, decreased through the stay-at-home period, and increased during reopening. Hydroxychloroquine, a drug publicized for its potential to treat COVID-19, had elevated concentrations in the week following the implementation of the United States Emergency Use Authorization. Our results relate to nationwide reports of increased demand for fentanyl, antidepressants, and other medications, as well as increased drug overdose deaths during the pandemic. Though wastewater surveillance during the pandemic has largely focused on measuring SARS-CoV-2 RNA concentrations, chemical analysis can also show trends that are important for revealing the public and environmental health effects of the pandemic.

Key words: wastewater, COVID-19, high resolution mass spectrometry, suspect screening, pharmaceuticals and personal care products

Synopsis: Sewage sludge provides evidence of changes in illicit drug, pharmaceutical, and household chemical use during the COVID-19 pandemic.

TOC Art


Antidepressants
Concentration
Time
Opioids
Concentration
Time

Introduction

The COVID-19 pandemic has dramatically increased the practice of wastewater-based epidemiology, with scientists and public health practitioners worldwide monitoring levels of SARS-CoV-2 RNA in untreated wastewater (1). Measurements of SARS-CoV-2 in wastewater and sludge are associated with daily case rates from testing and COVID-19 related hospitalizations, and can provide early information about potential clusters and outbreaks of COVID-19 (2, 3). Historically, wastewater-based epidemiology has focused primarily on chemical contaminants, which can provide information about the habits of the population within the catchment area of a treatment plant. Chemical analysis of wastewater has been used to track use of licit and illicit drugs and pharmaceuticals such as antidepressants, benzodiazepines, opioids and asthma medications, as well as exposure to pesticides and plasticizers (4–6). Wastewater analysis can be a highly efficient way to gather information about topics such as use of illegal drugs and psychoactive medications, without identification of individual persons. Additionally, wastewater analysis has been used to track antiviral and antibiotic use during influenza pandemics throughout the world (7–9).

The COVID-19 pandemic has affected many aspects of daily life beyond the direct effects of the virus, and we hypothesized that these changes would be visible in the organic chemical signature of wastewater. Our objectives were to characterize temporal variation of chemical contaminants in sewage sludge during the COVID-19 outbreak and associated lockdown and to relate our findings to the health and activities of local residents as well as broader global trends. We used both targeted and suspect screening methods to cover a broad range of contaminants including common analytes such as pharmaceuticals and illicit drugs (4), but also more unusual compounds for wastewater epidemiology studies such as disinfectants, UV-filters, and pesticides. Previous studies evaluating chemical concentrations in wastewater during the COVID-19 pandemic have focused on limited numbers of analytes – primarily licit and illicit drugs (10–14). Additionally, to our knowledge, this study is the first to report trends in wastewater concentrations for chemicals with direct significance to the COVID-19 pandemic including hydroxychloroquine and disinfectants. Samples were taken at the East Shore Water Pollution Abatement

Facility, New Haven, CT USA, where SARS-CoV-2 concentrations and cased data have already been measured and published (2). Daily collection of primary sludge samples and analysis for SARS-CoV-2 RNA began March 19, 2020 and has continued through 2020 (2).

Materials and Methods

Primary sludge samples were collected daily from March 19 to June 30, 2020 between 8 and 10 am at the East Shore Water Pollution Abatement Facility, New Haven, CT USA, as described in Peccia et al., 2020 (2). This treatment plant serves an estimated population of 200,000 in New Haven, Hamden, East Haven, and Woodbridge, CT, USA, and part of the service area contains combined sewers. Samples included both liquid and solid fractions (2 to 5% solids wt/wt) of sludge and were stored at -80°C until analysis. We analyzed daily samples from March 19 to April 15, and weekly composite samples from March 19 to June 30. Weekly sample extracts were further combined into 5-week composite samples, which were used for compound identification analysis only. **Figure 1** shows the sampling timeline relative to key dates for the pandemic and related shut down.

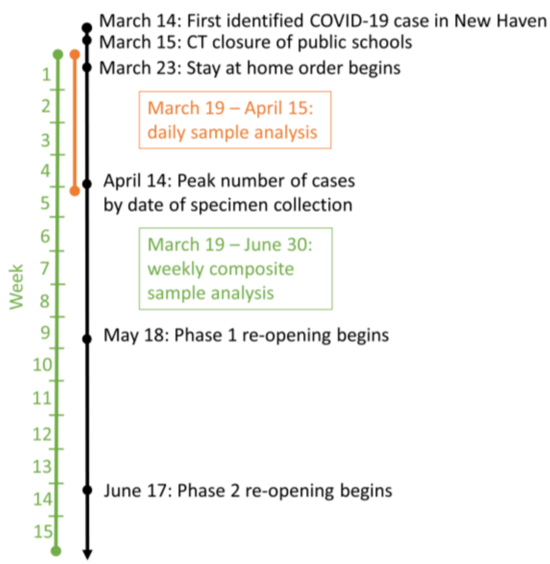


Figure 1. Timeline showing key pandemic related events and the timing of sample collection. We analyzed daily samples for four weeks during the initial increase in local COVID-19 cases. We analyzed weekly composite samples for a total of 15 weeks which covered the early stages of the pandemic and shut down as well as the initial stages of re-opening. All dates are within the year 2020.

Our analytical approach was based on long-term in-house methods used on food samples and other matrices. Our goal was to detect a broad range of contaminants. As we did not know what chemicals were present prior to sample analysis, we opted for minimal sample processing to avoid removing any unknowns.

Briefly, liquid and solid fractions were separated via centrifugation. Solids were extracted with acetonitrile, and equal amounts of the liquid fraction and acetonitrile extract were combined and filtered (method and materials details and recovery information available in sections S.1.1, S.1.2, and S.2.1). This type of method leads to complex sample matrix that requires high analytical sensitivity and selectivity, which are provided by the chosen instrumentation.

Samples were analyzed using an Ultimate 3000 liquid chromatograph coupled with a Q-Exactive mass spectrometer (Thermo Scientific) and positive electrospray ionization. Mobile phases were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile. We used an Agilent SB-C18 RRHD 1.8 µm, 2.1 x 150 mm column and a 55-minute method with a gradient of 5% B to 95%B. Calibration points, blanks, and daily, weekly, and 5-week composite samples were analyzed using an alternating full MS and all ion fragmentation (AIF) method. Additionally, the 5-week composite samples were analyzed using data dependent MS2 (ddMS2) analysis with an iterative inclusion approach, which has similar advantages to previously reported intelligent acquisition methods (15, 16). Briefly, we used the full scan data to generate inclusion lists including all features after blank filtering to ensure ddMS2 spectra were collected for each peak in the three 5-week composite samples. Each 5-week composite was injected 10 or 11 times, each run with a separate inclusion list for ddMS2 data collection. Additional instrument method and iterative inclusion information is in sections S.1.2-3 and S.2.3.

We used three separate data processing methods to identify and (semi-)quantify compounds in the samples. Full method descriptions, confidence levels for compound identification, and information on accuracy and variability are provided in sections S.1.4-7, S.2.1, and S.2.4. First, we used a targeted approach with TraceFinder software version 4.1 (Thermo Scientific) to conduct quantitative analysis based on standards for 62 compounds (listed in Table S1). Analytes included a variety of toxins, pharmaceuticals and illicit drugs known to be found in wastewater and/or sludge, and several compounds chosen for their relevance to COVID-19 treatment and prevention. Concentrations in the sludge extracts were determined based on a calibration curve that ranged from 0.1 ng/mL to 100 ng/mL. We used a separate method in

TraceFinder to screen our data using an in-house database of approximately 1800 compounds. The database contains exact MS1 and MS2 masses and retention times for many compounds that have previously been measured in house or by collaborators with the same (or very similar) instrument methods used in this project. The database also contains MS1 and MS2 masses that are provided in the Thermo Scientific EFS_HRAM database in TraceFinder (without retention times). Compound identifications using the screening method were based on exact mass matches for MS1 and MS2 masses, isotope pattern matching, and retention time matching where available. Only the Full MS/AIF data was used in the TraceFinder methods. The third method used Compound Discoverer version 3.1 software (Thermo Scientific), and identified compounds based on the ddMS2 data for the 5-week composite samples and spectral matches with the mzCloud database. The full MS data for the daily and weekly samples was then screened for the identified compounds. Peak areas were used for semi-quantitative trend analysis for the compounds identified with Compound Discoverer and TraceFinder screening methods. Each identification was assigned a confidence level based on available evidence. In the main text, identifications based on analytical standards are referred to as "confirmed" while confident screening results (from TraceFinder and Compound Discoverer) are "probable" and screening results where more ambiguity remains are listed as "tentative" (17). More information, including detailed, software specific confidence levels for each identification, is available in sections S.1.4-7, and section S.2.2.

Trends over time for each identified compound in daily and weekly samples were determined using two types of analysis: linear regression and multigroup analysis. Multigroup statistical tests used were determined based on the normality and homoscedasticity of each dataset. Trends listed as "increase" in **Table 1** indicate a statistically significant positive linear regression ($p \leq 0.05$) or a multigroup analysis where there were statistically significant differences between groups ($p \leq 0.05$) and an increase in average compound levels in the sludge. Trends listed as "decrease" in **Table 1** indicate a statistically significant negative linear regression ($p \leq 0.05$) or a multigroup analysis where there were statistically significant differences between groups ($p \leq 0.05$) and a decrease in average compound levels in the sludge.

Concentrations based on an external calibration curve were used for trend analysis where available (for a portion of the "confirmed" compounds); peak area was used for all other trend analyses (for all other compounds). Detailed statistical methods and results for trend determination are available in sections S.1.8 and S.2.2. **Table 1** also includes the relative standard deviation (RSD) of each compound concentration or peak area (from replicate unspiked samples, $n \geq 3$) as an estimate of measurement error.

Ten additional standards were purchased and analyzed after data analysis took place in an effort to improve annotation confidence for interesting results. We found that 9 of 10 compounds were correctly identified (amitriptyline, citalopram, diphenhydramine, triclocarban, didecyldimethylammonium, acetaminophen, benzotriazole, sertraline, and oxybenzone). Results for these compounds are reported as "confirmed", but trend analysis is based on peak area due to lack of quantitative standards run alongside the samples. The misidentified compound is not included in our results. Detailed quality control and methodological results are available in sections S.2.1, S.2.3, and S.2.4.

Results and Discussion

We identified chemicals in wastewater primary sludge and analysed their trends over time in daily samples from March 19 to April 15, 2020, and weekly composite samples from March 19 to June 30, 2020. Compound identifications were performed using both targeted and non-targeted strategies, and each compound was assigned a confidence level. **Table 1** shows the full list of identified compounds, their uses, their detection information, and the observed trends over time. Trends in identified compounds are discussed categorically below.

Table 1: Compounds identified in daily and weekly sludge samples

	Compound	Use	Confidence Level	Trends: Daily Samples (3/19/20-4/15/20)	Trends: Weekly Samples (3/19/20-6/30/20)	*m/z* measured[a]	Δ mass (ppm)[a,b]	Retention Time (min)[a]	RSD[c]
COVID-19 drugs and disinfectants	Hydroxychloroquine	antiviral	Confirmed	increase[d]		336.1835	-0.72	6.17	9
	Azithromycin	antibiotic	Confirmed		decrease	749.5152	-0.74	12.58	5
	Acetaminophen	analgesic	Confirmed		increase	152.0706	-0.28	5.22	7
	Triclocarban	disinfectant	Confirmed	increase		314.9849	-1.34	32.98	35
	Didecyldimethylammonium	disinfectant	Confirmed			326.3778	-0.86	40.98	60
	Cetrimonium	disinfectant	Probable			284.3308	-1.18	38.56	46
	Dioctyldimethylammonium	disinfectant	Probable		increase	270.3154	-0.64	37.8	73
	Dodecyltrimethylammonium (A)	disinfectant	Tentative			228.2685	0.11	30.88	45
	Dodecyltrimethylammonium (B)	disinfectant	Tentative		increase	228.2686	0.15	27.32	15
Opioids and Drugs of Abuse	Fentanyl	opioid	Confirmed		increase	337.2273	-0.45	16.06	25
	Levorphanol	opioid	Confirmed	decrease	decrease	258.1853	0.03	10.2	19
	Methadone	opioid	Confirmed		increase	310.2164	-0.45	20.3	17
	Codeine	opioid	Confirmed			300.1594	-0.17	6.18	2
	Hydromorphone	opioid	Confirmed	--[e]	increase	286.1439	0.53	4.05	9
	Oxycodone	opioid	Confirmed	--[e]		316.1543	-0.22	7.07	5
	Tilidine	opioid	Probable			274.1791	-3.71	41.26	24
	Tramadol	opioid	Probable			264.1957	-0.32	10.18	11
	Cocaine	cocaine	Confirmed		increase	304.1542	-0.35	12.16	6
	Benzoylecgonine	cocaine	Probable		increase	290.1386	-0.43	9.54	10
	Ecgonine methyl ester	cocaine	Probable		increase	200.1278	-1.38	2.30	28
	Anhydroecgonine	cocaine	Probable		decrease	168.1019	-0.25	7.08	20

	THC	cannabis	Probable		decrease	315.2315	-1.20	40.67	31
	Cannabidiol[f]	cannabis	Probable			315.2315	-1.2	36.81	27
	11-Hydroxy-δ(9)-THC	cannabis	Probable			331.2264	-1.11	33.25	13
	Nor-9-carboxy-9-THC	cannabis	Probable			345.2059	-0.45	33.53	22
	THC-A	cannabis	Tentative	increase	increase	359.2211	-1.70	42.66	27
	Methamphetamine	amphetamine	Confirmed			150.1277	-0.08	7.49	13
	TFMPP	party drug	Tentative		decrease	231.1106	1.01	2.00	46
Antidepressant and Antiseizure Drugs	Doxepin	antidepressant	Confirmed		increase	280.1696	-0.16	17.04	25
	Amitriptyline	antidepressant	Confirmed		increase	278.1903	-0.1	20.49	19
	Citalopram	antidepressant	Confirmed		increase	325.171	-0.31	17.4	17
	desmethyl-citalopram	antidepressant	Probable		increase	311.1553	-0.47	17	10
	Sertraline	antidepressant	Confirmed	increase		306.081	-0.3	21.47	10
	Trazadone	antidepressant	Probable			372.1584	-0.44	14.87	19
	Venlafaxine	antidepressant	Probable			278.2114	-0.15	14.28	18
	Clozapine	antipsycotic	Probable		increase	327.137	-0.26	14.3	22
	Carbamazepine	anticovulsant	Probable			237.1022	-0.8	18.93	11
	Gabapentin	anticonvulsant	Probable			172.1331	-0.5	6.89	4
	Pregabalin	anticonvulsant	Tentative			160.133	-1.11	1.99	5
Pharmaceuticals - other	Propafenone	antiarrythmic	Probable			342.2061	-0.8	34.23	23
	Trimethoprim	antibiotic	Probable			291.1450	-.63	8.02	8
	Diphenhydramine	antihistamine	Confirmed		increase	256.1695	-0.43	17.04	19
	Fexofenadine	antihistamine	Probable			502.295	-0.36	20.53	12
	Raltegravir	antiviral	Probable			445.1629	-0.32	20.87	12
	Darunavir	antiviral	Probable			548.2424	-0.13	24.21	5
	Zalcitabine	antiviral	Tentative		decrease	212.1027	-.130	2.02	8
	Losartan	ARB inhibitor	Confirmed	decrease	decrease	423.1693	-0.4	20.47	7
	Valsartan	ARB inhibitor	Probable			436.2341	-0.42	25.38	18

	Atenolol acid	beta-blocker	Probable			268.1542	0.6	7.79	5
	Carvedilol	beta-blocker	Probable			407.1963	-0.5	19.19	17
	Labetalol	beta-blocker	Probable			329.1858	-0.4	14.33	23
	Metoprolol	beta-blocker	Probable			268.1906	-0.33	11.55	50
	Propranolol	beta-blocker	Probable			260.1645	-0.08	15.69	44
	Verapramil	blood pressure	Probable			455.2902	-0.48	20.6	22
	Warfarin	blood thinner	Probable			309.1120	-0.42	24.72	22
	Metformin	diabetes	Tentative			130.1086	-0.76	1.83	7
	Raloxifine	estrogen regulator	Probable			474.1733	-0.1	17.41	51
	Cinchophen	gout	Probable		increase	250.086	-0.89	42.24	18
	Cyclobenzaprine	muscle relaxant	Probable			276.1746	-0.16	19.76	22
	Tolycaine	pain - injection	Probable		decrease	279.1702	-0.52	13.02	28
	Pramocaine	pain - topical	Probable		increase	294.2063	-0.2	18.77	18
	Edaravone	stroke and ALS	Probable	decrease	decrease	175.0865	-0.25	10.59	40
	Berberine	supplement	Confirmed			336.1229	-0.44	16.17	20
	Piracetam	supplement	Tentative			143.0814	-1.03	1.90	12
	Betanechol	urinary retention	Tentative	decrease	decrease	161.1283	-0.72	1.71	7
Personal Care Products	Oxybenzone	UV-filter	Confirmed	decrease	increase	229.0859	0.06	29.96	16
	Avobenzone	UV-filter	Probable		increase	311.1636	-1.92	41.52	28
	Octocrylene	UV-filter	Probable		increase	362.2111	-1.01	42.25	18
	Galaxolidone	fragrance	Tentative			273.1847	-0.79	35.95	15
	Nicotine	tobacco	Probable			163.1228	-1.36	2.16	11
	Caffeine	stimulant	Probable		increase	195.0876	0.16	7.81	5
Other chemicals	Benzotriazole	anti-corrosion	Confirmed	decrease	increase	120.0559	2.08	9.51	5
	Levamisole	veterinary drug	Probable			205.0793	-0.66	7.48	44
	Ipronidazole	veterinary drug	Tentative	decrease	decrease	170.0922	-1.08	1.71	4
	Imazalil	pesticide	Probable	increase	decrease	297.0555	-0.26	18.67	10

	Piperonyl-butoxide	pesticide	Probable	decrease		356.2427	-1.35	35.60	24
	Dinotefuran-metabolite-UF	pesticide	Tentative	--[e]		159.1126	-1.33	1.83	10
	Nithiazine	pesticide	Tentative		decrease	161.0377	-1.28	1.90	22

[a] Detailed description provided in section S.2.1

[b] Difference from theoretical *m/z*

[c] Relative standard deviation of concentration or peak area for replicate extractions of an unspiked sample (n=3 or n=6)

[d] Elevated in week 3 only

[e] Multidirectional changes in multivariate analysis

[f] In daily (but not weekly) solvent blanks at high levels

COVID-19 drugs and disinfectants.

In the early days of the pandemic the drug combination of hydroxychloroquine and azithromycin received consideration as a potential treatment for COVID-19. The US FDA issued an emergency use authorization (EUA) on March 28, 2020 (week 2 of our data), which remained in effect until June 15, 2020 (week 13) (18). As shown in **Figure 2a**, hydroxychloroquine concentrations increased in daily sludge samples in the third week of our study. While an overall hydroxychloroquine trend was not observed during the time that weekly samples were collected, a clear increase in concentration occurs in week 3 (**Figure 2b**). Hydroxychloroquine has an elimination half-life in the human body of approximately 22 days for oral doses and over 40 days for intravenous doses (19, 20), thus the increase in sludge concentrations is not as immediate or drastic as it would be for a drug with a shorter half-life. Our data indicates that the EUA and the large amount of publicity generated around hydroxychloroquine had significant impact on the amount used in the New Haven area, which includes two major hospitals. Hydroxychloroquine is normally used to treat malaria, lupus and rheumatoid arthritis (20), which are unlikely to have changed during the pandemic. Azithromycin concentrations decreased over the study period (weekly samples, **Figure 2b**). Azithromycin is only sometimes used in combination with hydroxychloroquine (21) and is more frequently used to treat bacterial respiratory infections which typically decline in the spring (22). Acetaminophen, which can be used to treat COVID-19 symptoms such as fever and headache, had limited availability during the pandemic, likely due to increased demand (23). Correspondingly, acetaminophen sludge concentrations increased in our weekly sample analysis (**Table 1,** Table S8).

Disinfectant use for cleaning both hands and surfaces has grown during the pandemic (24). Previous studies have shown pandemic related increases in concentrations of quaternary ammonium disinfectants in household dust (25), and higher risk of health effects due to increased exposure (26). Levels of two quaternary ammonium disinfectant chemicals increased in sludge during the overall study period (weekly samples, **Figure 1d,** Table S8). Triclocarban, an antibacterial compound used in consumer and medical grade handwashes increased in concentration in our daily sampling period (**Figure 1c**). Triclocarban was previously banned in medical grade hand washes (2017) and rubs and consumer hand washes (2016) for its endocrine disruption potential and other negative health effects (27–29). However, the most recent ruling against triclocarban (regarding consumer antiseptic rubs) took place in 2019, with an effective date of April 13, 2020 (30). Thus, it is likely that triclocarban products use had not yet been fully phased out during our study period. Additionally, the pandemic is likely to have prompted increased use of soaps and hand sanitizers that were previously stored. We identified an additional 3 disinfectant compounds for which there were no trends detected during the study period (**Table 1**).

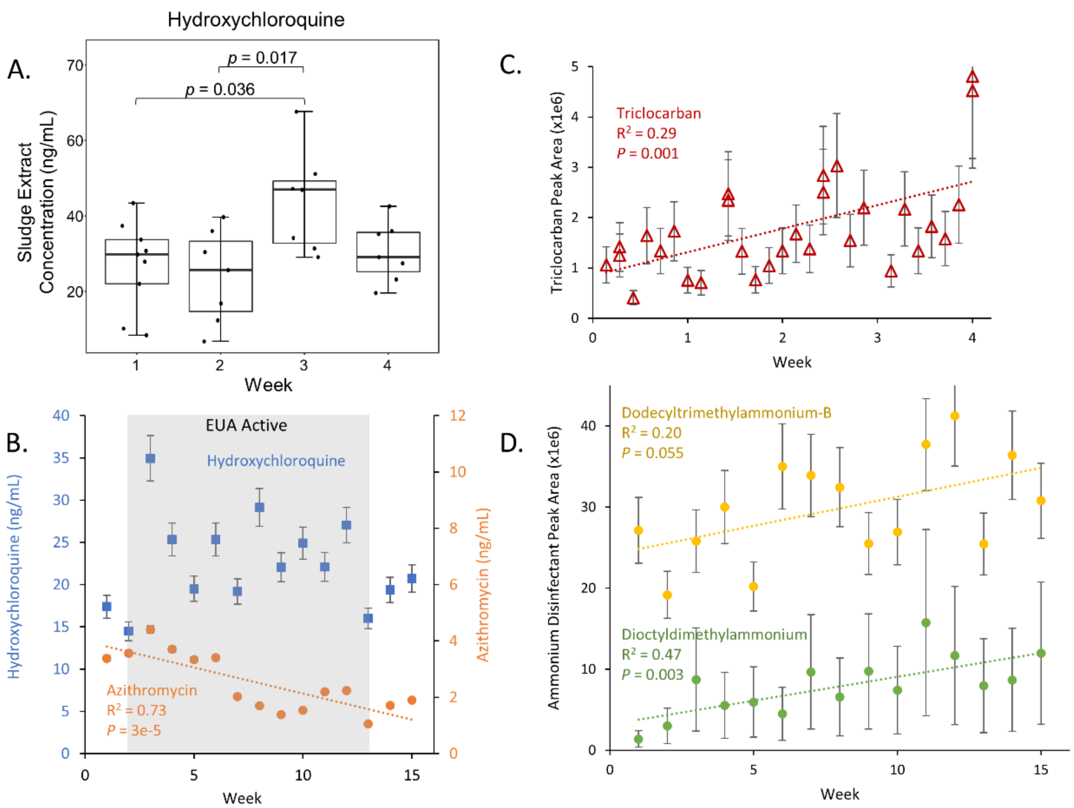


Figure 2. Trends for COVID-19 related drugs and disinfectants detected in daily and weekly primary sewage sludge samples. (A) boxplot showing a significant increase in hydroxychloroquine concentrations in week 3 samples based on daily sample concentrations (ANOVA with Tukey's HSD post-hoc analysis). (B) Scatter plot showing hydroxychloroquine and azithromycin concentrations in weekly composite samples. (C) Scatter plot showing increasing triclocarban levels in daily sludge samples. (D) Scatterplot showing data for two quaternary ammonium disinfectants in weekly composite sludge samples. Though $p > 0.05$ for dodecyltrimethylammonium-B, our multi group analysis showed a significant trend (Table S8). All scatterplot error bars show the RSD for each compound, calculated from one set of replicate samples.

Opioids and drugs of abuse

The ongoing epidemic of opioid abuse across the US has included the State of Connecticut (31). Additionally, there are pandemic-related increases in legal use of opioids; in April of 2020, the U.S. Drug Enforcement Agency authorized increased production quotas for fentanyl, morphine, hydromorphone, codeine to meet COVID-19 treatment needs, as well as for methadone, to ensure addiction treatment centers are adequately supplied (32). Sludge concentrations of fentanyl, methadone, and hydromorphone increased during our study period (weekly samples, **Figure 3a**). Fentanyl and methadone are commonly used both legally and illegally. Hydromorphone is itself a drug, but it is also a metabolite of morphine, codeine, and other opioids, thus its increasing levels are an indication of overall increase in opioid concentrations (33). Levorphanol, an opioid used for pain management and as a preoperative drug (34), decreased in both daily and weekly sludge samples (**Figure 3a, Table 1**). This decrease is potentially due to the reduction in elective procedures during the study period (35). We did not observe trends over time for an additional four opioids (**Table 1**). We note that our method was not capable of measuring heroin at these low concentrations (section S.2.1).

Concentrations of cocaine and two of its metabolites (ecgonine methyl ester and benzoylecgonine) also increased in the weekly samples (**Figure 3b,** Table S8). Anhydroecgonine, a metabolite specific for crack cocaine (36), decreased in the weekly samples, suggesting the possibility of a shift in local cocaine use patterns (**Figure 3b**). We saw no trends for methamphetamine, though the party drug TFMPP decreased during the study period (**Table 1**, Table S8). Cannabis related compounds did not show a consistent trend. Interestingly THC-A, the non-psychoactive precursor to THC found in raw plant material increased, whereas THC (transformed from THC-A by decarboxylation during heating above 105ºC for example in cooking or smoking) decreased across the study period (**Table 1**, Table S8).

The pandemic has increased risk factors for the development of substance abuse disorders and overdoses, such as isolation and economic distress. High COVID-19 related worry has been shown as a predictor of beginning substance use during the pandemic (37), and increasing numbers of overdoses have been reported nationwide (38). An increase in the amount of emergency responses necessary for opioid overdoses has occurred in some locations (39). Locally, there were 36 fatal overdoses during the study period in the towns/cities served by the East Shore Water Pollution Abatement Facility in New Haven (New Haven, East Haven, Woodbridge, and Hamden) (40). Thirty-two of these overdoses involved opioids, including 28 where fentanyl was detected. Cocaine was involved in 17 of the overdose deaths. Most cases included multiple drugs (40). Additionally, the COVID-19 pandemic has caused many changes in treatments for both pain and substance abuse disorders, which usually depend heavily on in-person interactions and carefully controlled access to medications. New systems for opioid distribution and telemedicine appointments have been developed but there is continued concern over their effectiveness (41–43).

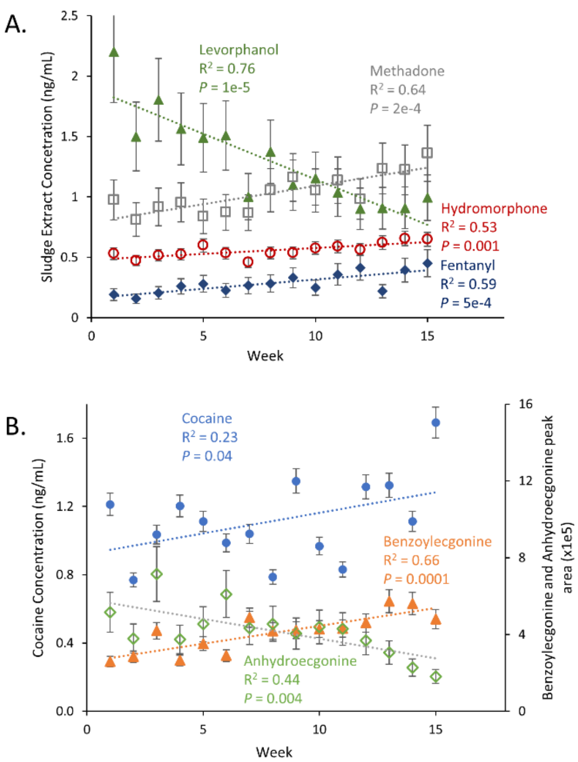


Figure 3. Trends for opioids and cocaine related compounds detected in weekly composite primary sewage sludge samples. (A) Scatter plot showing opioid concentrations. (B) Scatter plot showing levels of cocaine and cocaine metabolites. All scatterplot error bars show the RSD for each compound, calculated from one set of replicate samples.

Antidepressants and other medications

Many people have struggled with mental health challenges during the COVID-19 pandemic and incidence of depression has increased in the US during the pandemic (44). Additionally, there is evidence that people with psychiatric disorders are at increased risk for COVID-19 infection (45), and that COVID-19 infection is associated with new diagnoses of psychiatric illnesses (46). Increased demand for the antidepressant drug sertraline has caused shortages throughout the U.S. (47, 48). Sertraline levels increased in our analysis of daily sludge samples (**Figure 4a)**. In our weekly sample analysis, the levels of three additional antidepressants (citalopram, amitriptyline, and doxepin), one antidepressant metabolite (desmethylcitalopram), and the antipsychotic drug clozapine increased (**Figure 4b, Table 1,** Table S8). No trend was observed for an additional 3 antidepressants and 3 anticonvulsant drugs (**Table 1**, Table S8).

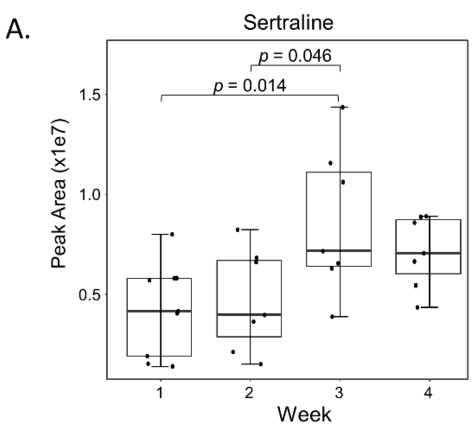
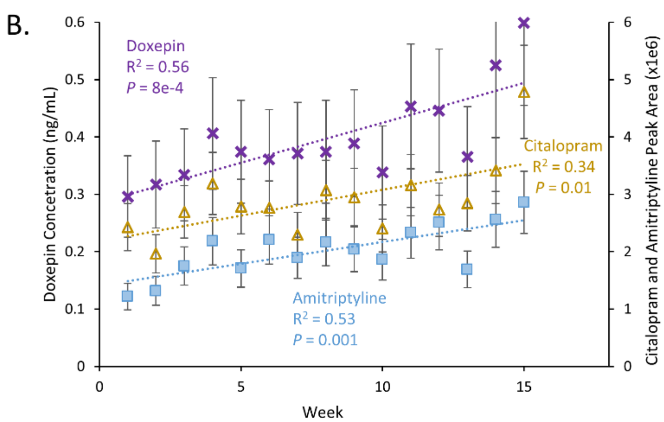


Figure 4. Trends for antidepressants detected in daily and weekly primary sewage sludge samples. (A) Boxplot showing a significant increase in sertraline during the 4 weeks of daily sampling (ANOVA with Tukey's HSD post-hoc analysis). (B) Scatter plot showing doxepin, citalopram, and amitriptyline levels in weekly composite samples. Scatterplot error bars show the RSD for each compound, calculated from one set of replicate samples.



We also observed various trends for other pharmaceuticals identified in our analysis (**Table 1,** Table S8, Figures S3-S5). Some of these trends are likely related to pandemic-induced changes in behaviour, while others are not. For example, tolycaine, a local anaesthetic used in dental injections (49), decreased in the sludge samples, which corresponds to a decrease in dental appointments during the shutdown (50). Pramocaine, a mild anaesthetic used in over-the-counter creams (51), had increasing levels in sludge which is more likely due to seasonal changes in exposure to insect bites and poison ivy than to pandemic related changes. Diphenhydramine, an allergy medication, also increased during the study period (**Table 1**, Table S8).

Personal care product ingredients and other chemicals

We found that benzotriazole, a corrosion inhibitor frequently used on cars and a known contaminant in road dust (52), had trends in sludge that corresponded to the shut down and phase one reopening that occurred during our study period (**Figure 5a**). There was a decrease in the daily and weekly composite sample concentrations at the beginning of the study period, and then an increase in weekly composite sample levels starting in the weeks before Phase 1 reopening. We hypothesize that the benzotriazole trends are due to changes in the amount of traffic. Doucette et al., found that traffic in Connecticut decreased 43% during the stay-at-home order that began in the first week of our study period (53), and air pollutants related to traffic decreased during stay-at-home orders in other locations (54, 55). With fewer cars on the road, less benzotriazole washes off cars onto the road, and thus less is dissolved the in the runoff water that enters the combined sewer system. Benzotriazole is also used on aircrafts as a de-icer and corrosion inhibitor (56). There is one small airport in the study area that, like many other airports, experienced decreased traffic during the stay-at-home order. Benzotriazole is also used in household dishwasher detergents, which is likely a smaller source to combined sewer wastewater systems.

All the UV-filter compounds detected increased in the weekly composite samples (**Figure 5b**). This trend is likely due to the increase in sunscreen use that corresponds to the seasonal change that occurs in Connecticut between March and June. A slight decrease in oxybenzone levels was observed in the daily samples and the first weekly samples which may be reflective of decreased cosmetic usage during the stay at home order while there was still wintery weather. We suspect that the other trends we found in this category were not affected by the pandemic or stay at home order (**Table 1,** Table S8).

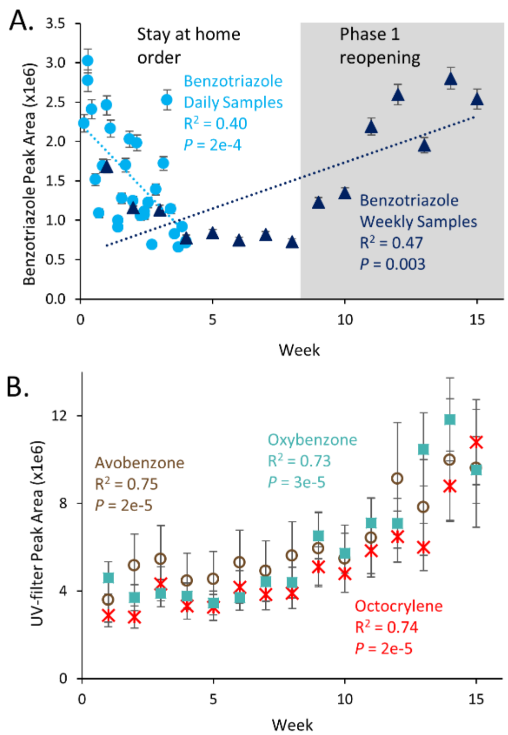


Figure 5. Trends for additional chemicals detected in daily and weekly primary sewage sludge samples. (A) Scatterplot showing benzotriazole levels in daily and weekly samples. (B) Scatter plot showing UV-filter levels in weekly composite samples. Scatterplot error bars show the RSD for each compound, calculated from one set of replicate samples (n = 6).

Broader relevance, limitations, and future directions

Though our results are specific to the New Haven, CT area, many of the trends that we found are more broadly relevant. We observed increased concentrations for medications whose demand increased during the pandemic (47) and increasing trends for illegal drugs that align with the increasing number of overdoses nationwide (38). Wastewater monitoring can be a way to monitor drug usage during this time when other monitoring strategies have been disrupted by the pandemic (57, 58). Moreover, if wastewater trends can be associated with public heath monitoring data, wastewater-based information can play an important role in providing real-time estimates or early warnings of a variety of infectious and non-infectious disease. We note that our results on drugs of abuse differ from those reported by wastewater monitoring programs in Europe, where there has been an overall decrease in illicit drug use (10). Specifically, a study in Austria found decreased use of cocaine, amphetamine, and MDMA during the initial COVID-19 lockdown, which were partially compensated for by increased methamphetamine use (13). They saw no changes in cannabis or methadone related compounds relative to other years (13). Additionally, wastewater monitoring and drug use surveys in Australia have revealed record low levels of fentanyl and oxycodone, but regional increases in cocaine, heroin, methamphetamine, and cannabis (11). The differing trends may be related to differences in pandemic severity and local political responses, but are also reflective of existing trends from before COVID-19; the opioid crisis that is prominent throughout the US has not affected Australia nor Europe to the same extent (10, 11). Trends we observed for pharmaceuticals are more similar to those reported by the Austrian study; though there is some variation individual compound results, both studies show consistent levels of long term medications such as beta-blockers and anticonvulsants and lowered levels of short term medications such as analgesics and pharmaceuticals (13).

In addition to human health related trends, our results also reveal trends in chemical releases that may affect the environment. Though our samples did not undergo the complete wastewater treatment process, many of the compounds we detected are not fully removed by standard treatment trains (59–61) and are released with the effluent water or sewage sludge. We detected endocrine disrupting compounds including triclocarban, oxybenzone, and sertraline that can have negative impacts on marine organisms and cycle back to humans via consumption of local seafood (62, 63). Much concern has been expressed about the potential ecological impacts of increased pharmaceutical loads in wastewater, particularly in developing areas where wastewater treatment is limited and access to antibiotic and antiviral medications is not controlled by prescriptions (64–67). Spread of resistance to antibiotic and antiviral medications is also a potential concern (64, 67).

While our analytical method was designed to include a wide range of chemicals, the scope of any analysis is inherently limited. We intentionally included both liquid and solid portions of primary sludge to measure both hydrophilic and hydrophobic chemicals. However, this prohibited the exact quantification of chemicals in either phase. We therefore are not able to use our data to back calculate per capita consumption as has been done in other wastewater studies (4, 12, 13). Additionally, we designed our sample preparation method for the relatively small volume of sample available from corresponding research on levels of SARS-CoV-2 RNA in primary sludge; we could not use solid phase extraction to preconcentrate the liquid portion of our samples, as is common in wastewater studies (59, 60). This likely caused a decrease in the number of liquid phase contaminants we detected. Additionally, our unique method makes our quantitative results difficult to relate to other studies, though trends over time can still be compared. We note that our analytical methods were highly effective, and our sample collection and preparation method was simple, fast, and did not require specialized supplies. Sewage sludge is a well-mixed, concentrated source that does not require complex sampling equipment. Though we collected data over a relatively long period of time in 2020, our sampling campaign did not begin until the pandemic was underway; therefore, we cannot directly compare our results to those from previous years. The data presented in this manuscript represents only a small fraction of what was collected using our high-resolution mass spectrometry methods. We plan to conduct further investigation of chemicals in the sludge that were not easily identifiable using our databases and investigate chemical correlations with measured levels of SARS-CoV-2, as in Wang et al., 2020 who reported statistical relationships between a variety of chemical features and virus RNA levels (12).

Overall, the first wave of the COVID-19 pandemic and the related shut down had a significant influence on the chemical fingerprint of primary sludge in New Haven, CT. We found upwards trends in hydroxychloroquine and disinfectant concentrations in sludge, reflecting increased use during the initial wave of the COVID-19 pandemic. We also saw increases in drugs of abuse and antidepressants, and seasonal changes for chemicals such as UV-filters that are used in sunscreens. Importantly, we found that benzotriazole concentrations showed different trends during and after the local stay at home order, a key indication that benzotriazole can be used as a marker for the influence of traffic on wastewater and sludge in combined sewer systems. Overall, our findings relate strongly to trends in public and environmental health worldwide and show specific trends that may not have been picked up in other types of analysis. Sewage sludge surveillance is a promising strategy to monitor a variety human behavioural changes during the pandemic that have public health consequences.

Supporting Information

Supporting information is available for this manuscript that includes: information on materials and analytical standards; detailed sample preparation, instrumental analysis, data analysis, and statistical methods; QA/QC results for method performance; detailed confidence annotations and statistical results; results specific to iterative inclusion functionality and compound annotation accuracy.

Data sharing plans

This manuscript and associated SI has been uploaded to the pre-print server ChemRxiv (https://doi.org/10.26434/chemrxiv.13562525.v1). The .RAW instrument data files used in this study are available as a dataset on MassIVE (ftp://MSV000086676@massive.ucsd.edu) along with the full peak list produced in our Compound Discoverer analysis and the filtered peak list that includes only the compounds listed in this manuscript. Additional files including all TraceFinder data, the internal database used for suspect screening, and the R scripts used for statistical analysis are available from the authors upon request.

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References

1. G. Medema, L. Heijnen, G. Elsinga, R. Italiaander, A. Brouwer, Status of environmental surveillance for SARS-CoV-2 virus: Scientific brief. *Environmental Science and Technology Letters* **7**, 511–516 (2020).

2. J. Peccia, *et al.*, Measurement of SARS-CoV-2 RNA in wastewater tracks community infection dynamics. *Nature Biotechnology* **38**, 1164–1167 (2020).

3. G. Medema, L. Heijnen, G. Elsinga, R. Italiaander, A. Brouwer, Presence of SARS-Coronavirus-2 RNA in Sewage and Correlation with Reported COVID-19 Prevalence in the Early Stage of the Epidemic in the Netherlands. *Environmental Science and Technology Letters* **7**, 511–516 (2020).

4. P. M. Choi, *et al.*, Wastewater-based epidemiology biomarkers: Past, present and future. *Trends in Analytical Chemistry* **105**, 453–469 (2018).

5. N. I. Rousis, *et al.*, Wastewater-based epidemiology to assess pan-European pesticide exposure. *Water Research* **121**, 270–279 (2017).

6. I. González-Mariño, R. Rodil, I. Barrio, R. Cela, J. B. Quintana, Wastewater-Based Epidemiology as a New Tool for Estimating Population Exposure to Phthalate Plasticizers. *Environmental Science and Technology* **51**, 3902–3910 (2017).

7. A. C. Singer, *et al.*, Intra-and Inter-Pandemic Variations of Antiviral, Antibiotics and Decongestants in Wastewater Treatment Plants and Receiving Rivers. *PLOS one* **9**, e108621-undefined (2014).

8. T. Azuma, N. Nakada, N. Yamashita, H. Tanaka, Prediction, risk and control of anti-influenza drugs in the Yodo River Basin, Japan during seasonal and pandemic influenza using the transmission model for infectious disease. *Science of the Total Environment* **521–522**, 68–74 (2015).

9. Y. Zhang, *et al.*, Wastewater-based epidemiology in Beijing, China: Prevalence of antibiotic use in flu season and association of pharmaceuticals and personal care products with socioeconomic characteristics. *Environment International* **125**, 152–160 (2019).

10. European Monitoring Centre for Drugs and Drug Addiction, "Impact of COVID-19 on patterns of drug use and drug-related harms in Europe" (2020) (December 20, 2020).

11. Australian Criminal Intelligence Commission, "National Wastewater Drug Monitoring Program - Report 11" (2020).

12. S. Wang, *et al.*, High-throughput wastewater analysis for substance use assessment in central New York during the COVID-19 pandemic. *Environmental Science: Processes and Impacts* **22**, 2147–2161 (2020).

13. V. Reinstadler, *et al.*, Monitoring drug consumption in Innsbruck during coronavirus disease 2019 (COVID-19) lockdown by wastewater analysis. *Science of the Total Environment* **757** (2021).

14. R. Bade, *et al.*, Changes in alcohol consumption associated with social distancing and self-isolation policies triggered by COVID-19 in South Australia: a wastewater analysis study. *Addiction*, 1–5 (2020).

15. J. P. Koelmel, *et al.*, Expanding lipidome coverage using LC-MS/MS data-dependent acquisition with automated exclusion list generation. *Journal of the American Society for Mass Spectrometry* **28**, 908–917 (2018).

16. J. P. Koelmel, *et al.*, Towards comprehensive Per- and Polyfluoroalkyl Substances annotation using FluoroMatch Software and Intelligent High-Resolution Tandem Mass Spectrometry Acquisition. *Analytical Chemistry* **92**, 11186–11194 (2020).

17. E. L. Schymanski, *et al.*, Identifying small molecules via high resolution mass spectrometry: Communicating confidence. *Environmental Science and Technology* **48**, 2097–2098 (2014).

18. K. Thomson, H. Nachlis, Emergency Use Authorizations during the COVID-19 Pandemic: Lessons from Hydroxychloroquine for Vaccine Authorization and Approval. *Journal of the American Medical Association* **324**, 1282–1283 (2020).

19. S. Tett, D. Cutler, R. Day, K. Brown, Bioavailability of hydroxychloroquine tablets in healthy volunteers. *British Journal of Clinical Pharmacology* **27**, 771–779 (1989).

20. , Hydroxychloroquine | DrugBank Online (December 20, 2020).

21. E. S. Rosenberg, *et al.*, Association of Treatment with Hydroxychloroquine or Azithromycin with In-Hospital Mortality in Patients with COVID-19 in New York State. *Journal of the American Medical Association* (2020) https:/doi.org/10.1001/jama.2020.8630.

22. S. Coutu, L. Rossi, D. A. Barry, S. Rudaz, N. Vernaz, Temporal Variability of Antibiotics Fluxes in Wastewater and Contribution from Hospitals. *PLoS ONE* **8**, 53592 (2013).

23. K. Blankenship, Johnson & Johnson posts “temporary” Tylenol shortage amid heightened demand. *Fierce Pharma* (2020) (December 20, 2020).

24. P. I. Hora, S. G. Pati, P. J. McNamara, W. A. Arnold, Increased Use of Quaternary Ammonium Compounds during the SARS-CoV-2 Pandemic and Beyond: Consideration of Environmental Implications. *Environmental Science and Technology Letters* **7**, 622–631 (2020).

25. G. Zheng, G. M. Filippelli, A. Salamova, Increased Indoor Exposure to Commonly Used Disinfectants during the COVID-19 Pandemic. *Environmental Science and Technology Letters* **7**, 760–765 (2020).

26. D. Li, A. Sangion, L. Li, Evaluating consumer exposure to disinfecting chemicals against coronavirus disease 2019 (COVID-19) and associated health risks. *Environmental International* **145** (2020).

27. , Safety and Effectiveness of Consumer Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use. *Federal Register* **81**, 61106–61130 (2016).

28. , Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use. *Federal Register* **82**, 60474–60503 (2017).

29. R. U. Halden, *et al.*, The Florence Statement on Triclosan and Triclocarban. *Environmental Health Perspectives* **125**, 064501-undefined (2017).

30. , Safety and Effectiveness of Consumer Antiseptic Rubs; Topical Antimicrobial Drug Products for Over-the-Counter Human Use. *Federal Register* **84**, 14847–14864 (2019).

31. J. C. Allen, The Opioid Overdose Crisis in Connecticut. *Connecticut Medicine* **83**, 201 (2019).

32. , “DEA takes additional steps to allow increased production of controlled substances used in COVID-19 care” (2020) (December 20, 2020).

33. H. S. Smith, Opioid Metabolism . *Mayo Clinic Proceedings* **84**, 613–624 (2009).

34. , Levorphanol | DrugBank Online (December 20, 2020).

35. E. Stannard, With coronavirus slowing, hospitals performing elective surgeries. *New Haven Register* (2020) (January 11, 2021).

36. K. B. Scheidweiler, J. Shojaie, M. A. Plessinger, R. W. Wood, T. C. Kwong, Stability of Methylecgonidine and Ecgonidine in Sheep Plasma in Vitro. *Clinical Chemistry* **46**, 1787–1795 (2000).

37. A. H. Rogers, J. M. Shepherd, L. Garey, M. J. Zvolensky, Psychological factors associated with substance use initiation during the COVID-19 pandemic. *Psychiatry Research* **293** (2020).

38. A. Alter, C. Yeager, “COVID-19 Impact on US National Overdose Crisis” (2020) (December 20, 2020).

39. S. Slavova, P. Rock, H. M. Bush, D. Quesinberry, S. L. Walsh, Signal of increased opioid overdose during COVID-19 from emergency medical services data. *Drug and Alcohol Dependence* **214** (2020).

40. , Connecticut Office of the Chief Medical Examiner: Annual Statistics (2020) (December 20, 2020).

41. G. C. Alexander, K. B. Stoller, R. L. Haffajee, B. Saloner, An Epidemic in the Midst of a Pandemic: Opioid Use Disorder and COVID-19. *Annals of internal medicine* **173**, 57–58 (2020).

42. S. N. El-Tallawy, R. Nalamasu, J. v. Pergolizzi, C. Gharibo, Pain Management During the COVID-19 Pandemic. *Pain and Therapy* **9**, 453–466 (2020).

43. H. Shanthanna, *et al.*, Caring for patients with pain during the COVID-19 pandemic: consensus recommendations from an international expert panel. *Anaesthesia* **75**, 935–944 (2020).

44. C. K. Ettman, *et al.*, Prevalence of Depression Symptoms in US Adults Before and During the COVID-19 Pandemic. *JAMA network open* **3**, e2019686 (2020).

45. Q. Wang, R. Xu, N. D. Volkow, Increased risk of COVID-19 infection and mortality in people with mental disorders: analysis from electronic health records in the United States. *World Psychiatry*, 1–7 (2021).

46. M. Taquet, S. Luciano, J. R. Geddes, P. J. Harrison, Bidirectional associations between COVID-19 and psychiatric disorder: retrospective cohort studies of 62,354 COVID-19 cases in the USA. *The Lancet Psychiatry* (2020) https:/doi.org/10.1016/S2215-0366(20)30462-4.

47. US Food and Drug Administration, FDA Drug Shortages (2020) (December 20, 2020).

48. A. Edney, Zoloft in Short Supply as Prescriptions Soar During Pandemic. *Bloomberg* (2020) (January 5, 2021).

49. US National Institutes of Health, Tolycaine. *Inxight: Drugs* (January 5, 2021).

50. Connecticut Department of Public Health, “Best Practices for Dental Offices Considering Expanding Operations Beyond Emergency Care to Include Non-Urgent and Elective Procedures During the COVID-19 Pandemic” (2020) (January 5, 2021).

51. , Pramocaine | DrugBank Online (January 5, 2021).

52. J. Asheim, *et al.*, Benzotriazoles, benzothiazoles and trace elements in an urban road setting in Trondheim, Norway: Re-visiting the chemical markers of traffic pollution. *Science of the Total Environment* **649**, 703–711 (2019).

53. M. L. Doucette, *et al.*, Initial impact of COVID-19's stay-at-home order on motor vehicle traffic and crash patterns in Connecticut: An interrupted time series analysis. *Injury Prevention* **0**, 1–7 (2020).

54. J. Xiang, *et al.*, Impacts of the COVID-19 responses on traffic-related air pollution in a Northwestern US city. *Science of the Total Environment* **747** (2020).

55. I. Chowdhuri, *et al.*, Significant decrease of lightning activities during COVID-19 lockdown period over Kolkata megacity in India. *Science of the Total Environment* **747** (2020).

56. A. M. Sulej, Ż. Polkowska, J. Namieśnik, Z. Polkowska, J. N. Snik, Pollutants in Airport Runoff Waters. *Critical Reviews in Environmental Science and Technology* **42**, 1691–1734 (2012).

57. NIH National Institute on Drug Abuse, Monitoring the Future Study: Trends in Prevalence of Various Drugs | National Institute on Drug Abuse (NIDA) (2020) (January 5, 2021).

58. US Drug Enforcement Administration, DEA takes additional steps to allow increased production of controlled substances used in COVID-19 care (2020) (January 5, 2021).

59. M. S. Kostich, A. L. Batt, J. M. Lazorchak, Concentrations of prioritized pharmaceuticals in effluents from 50 large wastewater treatment plants in the US and implications for risk estimation. *Environmental pollution* **184**, 354–9 (2014).

60. J. Wang, S. Wang, Removal of pharmaceuticals and personal care products (PPCPs) from wastewater: A review. *Journal of Environmental Management* **182**, 620–640 (2016).

61. B. Petrie, R. Barden, B. Kasprzyk-Hordern, A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. *Water Research* **72**, 3–27 (2014).

62. B. S. Karthikeyan, J. Ravichandran, K. Mohanraj, R. P. Vivek-Ananth, A. Samal, A curated knowledgebase on endocrine disrupting chemicals and their biological systems-level perturbations. *Science of the Total Environment* **692**, 281–296 (2019).

63. L. Arpin-Pont, M. J. Martínez-Bueno, E. Gomez, H. Fenet, Occurrence of PPCPs in the marine environment: a review. *Environmental Science and Pollution Research* **23**, 4978–4991 (2016).

64. M. Usman, M. Farooq, K. Hanna, Environmental side effects of the injudicious use of antimicrobials in the era of COVID-19. *Science of the Total Environment* **745** (2020).

65. D. F. Farias, *et al.*, COVID-19 Therapies in Brazil: Should We Be Concerned with the Impacts on Aquatic Wildlife? *Environmental Toxicology and Chemistry* **39**, 2348–2350 (2020).

66. W. Espejo, J. E. Celis, G. Chiang, P. Bahamonde, Environment and COVID-19: Pollutants, impacts, dissemination, management and recommendations for facing future epidemic threats. *Science of the Total Environment* **747** (2020).

67. S. Horn, B. Vogt, R. Pieters, H. Bouwman, C. Bezuidenhout, Impact of Potential COVID-19 Treatment on South African Water Sources Already Threatened by Pharmaceutical Pollution. *Environmental Toxicology and Chemistry* **39**, 1305–1306 (2020).