Water Quality Changes within a Public Drinking Water Distribution System and in Private Homes; Before, During, and After a Free Chlorine Conversion

Maura J. Donohue[†]

[†] United States Environmental Protection Agency, Cincinnati, OH 45268.

2.0 Materials and Methods

2.4 Water quality measurements.

2.4.1Anion analysis (nitrate): A 100 µL sample was injected into a Dionex DX 500 IC system (Dionex Corporation, Sunnyvale, CA). The anions were separated on a Dionex AS9-HC (4 X 250 mm) analytical column using a gradient buffer of 9 mM sodium carbonate and MilliQ water. The instrument was calibrated daily, and standards and unknown samples were analyzed in the same batch. A standard curve was generated from the analyzed standards, and the chromatographic peaks of unknown samples were converted to mg/L for each anion. The LOD for nitrate as N was 0.05 mg/L.

2.5.2 Nontuberculous mycobacterium (NTM) culture.

2.5.2.1 DNA extraction for Sanger sequencing.

Single colonies from the primary isolation plate were selected for DNA extraction. Each colony was picked using a 10µl loop. The cell mass was placed into a 2 mL screw cap tube with 0.25g ±0.05, 0.1mm glass beads. A colony's DNA was extracted use the following extraction method. To each bead-beading tube, a 500µL aliquot of Tissue and Cell Lysis Solution (Lucigen

Corporation, Middleton, WI) was added. A bead-beater was used to lyse the cells (BioSpec Products, Bartlesville, OK). After a 5 min cool down in ice, the bottom of the tube was punctured with a 1/18 G needle (Becton, Dickerson and Company, Franklin Lakes, NJ). The bead beating tube was inserted into a sterile 1.5 mL microcentrifuge tube (Eppendorf, Hauppauge, NY), and the lysate was collected into the 1.5 mL microcentrifuge tube body by centrifugation at 3,500 rpm for 5 min. Next, 2 µL of Proteinase K (50 µg/µL) (Lucigen Corporation, Middleton, WI) was added followed by incubation at 65 °C in a water bath for 15 min. Next, 2 µL of RNase A (5 µg/µL) (Lucigen Corporation, Middleton, WI) was added to the mixture and incubated at 37 °C for 30 min. Subsequently, 350 µL of MPC Protein Precipitation Reagent (Lucigen Corporation, Middleton, WI) was added to precipitate the cellular proteins. The resulting supernatant was transferred to a sterile microcentrifuge tube containing an equal volume of ice-cold isopropanol (~ -4 °C). The samples were inverted manually up to 40 times and centrifuged at 10,000 x *g* for 10 min. The supernatant was poured off, and the resulting DNA pellet washed with 500 µL of ice cold (~ -4 °C) 70% ethanol. Samples were centrifuged, and the ethanol removed. The DNA pellet was air dried for 15 min to remove residual ethanol. The DNA pellets were re-suspended in 100 µL of nuclease-free sterile water and stored at -80 °C until analyzed. DNA extracts were quantified using a Nanodrop™ spectrophotometer (Thermo Scientific, Waltham, MA) and were subsequently stored at -80 °C.

2.5.2.2 Sanger sequencing PCR. The 16S rRNA gene was amplified by PCR and sequenced for subsequent isolate identification. Amplification of the 16S rRNA gene was performed with primers 8F and 1492R.(Lane, 1991) Amplification was done with a 25 µL reaction volume, containing 2.5 µL 10X PCR buffer, 1.25 µL of each 10 µM primer, 1.5–2.0 µL of 25 mM $MgCl_2$ solution, 2.0–2.5 µL of 10 mM dNTP's, 1 U/µL *Taq* polymerase, 13.1–13.3 µL dH_2O, and 2 µL

DNA. The thermocycle for each PCR reaction was an initial 5 min denaturation step at 95 °C, followed by an additional 30 sec at 95 °C. DNA was annealed at 50 °C for 60 sec with an extension for 30 sec at 72 °C plus a terminal 1 cycle at 72 °C for 5 min.

2.5.2.3 Sequence analysis and isolate identification. Genotyping-by-sequencing analysis of both forward and reverse PCR products was accomplished on an Applied Biosystems™ 3730*xl* DNA Analyzer using ABI's BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies, Grand Island, NY). Consensus sequences were generated on BioEdit Sequence Alignment Editor Version 7.1.3.0 for each gene using the forward and reverse sequence (Hall, 1999). The consensus sequences built in BioEdit were then transferred to Molecular Evolutionary Genetics Analysis (MEGA11) Version 11.0.13. for alignment, tree building and database searches (Tamura et al., 2021). National Center for Biotechnology Information's (NCBI) nucleotide non-redundant (nr) blast search was used to identify closely related sequences. NCBI blast search provided identity, percent homology, and accession number. This study's' unknown sequences were submitted to GenBank and were given the following accession numbers PO678713-PO678782. Next, a phylogenic tree (neighbor-joining) was created in MEGA11 using both reference NTM 16S sequences and this studies unknown sequences. The software compared NTM type strains 16S rRNA gene sequences to the unknown NTM isolates gene sequences.

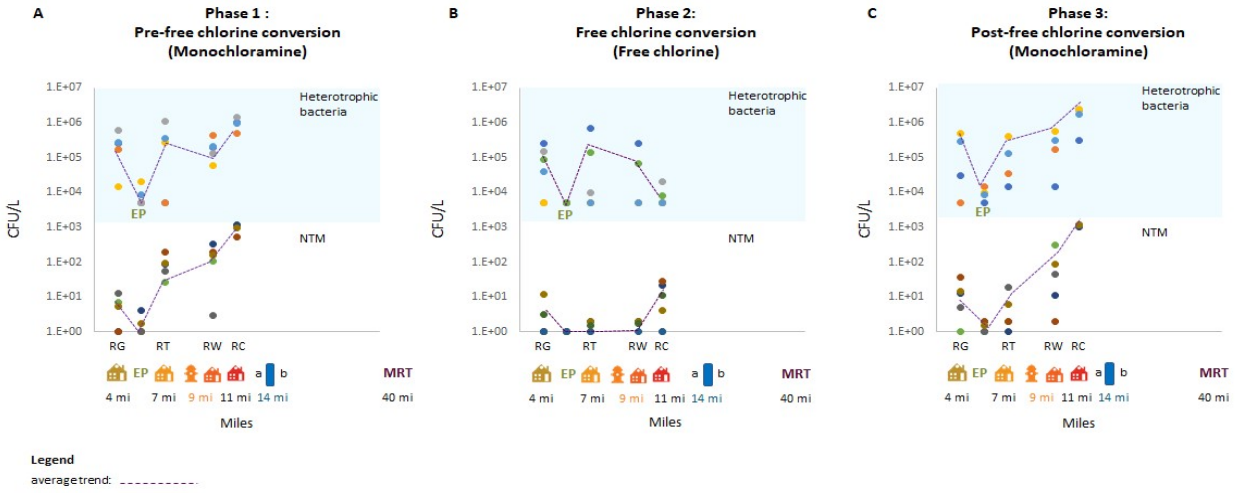
2.6 THM and HAA analysis.

Supplemental Table S1: THM 4 and HAA9 Limit of Detection

DBP Groups	Chemical name	Limit of Detection (LOD) µg/L
THM4	chloroform	0.05
	bromodichloromethane	0.05
	dibromochloromethane	0.05
	bromoform	0.05
HAA9	**trichloroacetic acid**	**1.0**
HAA5 bolded	**dichloroacetic acid**	**2.0**
	monochloroacetic acid	**2.0**
	dibromoacetic acid	**1.0**
	monobromoacetic acid	**1.0**
	bromochloroacetic acid	1.0
	bromodichloroacetic acid	1.0
	chlorodibromoacetic acid	2.0
	tribromoacetic acid	4.0

3.0 Result

3.2.



2 Disinfectant (residual) impact on microbial concentration.

Supplemental Figure S1: Scatter plots showing microbial concentrations measured at each home location. A) pre-free chlorine conversion, B) free chlorine conversion, and C) post-free chlorine conversion. The purple dotted line tracks the average microbial concentration at each location for heterotrophic bacteria and NTMs.

3.3.2 Assessing the relationship between disinfectant residual, and microbial trends in conjunction with nitrification parameters (free ammonia, nitrite and nitrate). Regression analysis was performed to estimate the relationship between HPC/NTM concentrations to free ammonia, nitrite, and nitrate levels. Based on the resulting R^2, free ammonia, nitrite, and nitrate are unimportant factors that did not impact HPC/NTM concentrations, Supplemental Table 3.

Supplemental Table S2: Regression analysis results (R^2) comparing free ammonia, nitrite, and nitrate concentrations to disinfectant residual (monochloramine/free chlorine and total chlorine) and microbe (heterotrophic bacteria and NTM) data for each of the three Phases.

R^2/P	Water Quality Parameter	Phase 1	Phase 2	Phase 3
Free ammonia	Total chlorine	$R^2 = 0.025$ $P = 0.562$	$R^2 = 0.000$ $P = 0.986$	$R^2 = 0.246$ $P = 0.051$
	Monochloramine /Free chlorine	$R^2 = 0.019$ $P = 0.614$	$R^2 = 0.001$ $P = 0.882$	$R^2 = 0.284$ $P = 0.034$
	Heterotrophic bacteria	$R^2 = 0.027$ $P = 0.403$	$R^2 = 0.138$ $P = 0.106$	$R^2 = 0.467$ $P = 0.003$
	NTM	$R^2 = 0.016$ $P = 0.638$	$R^2 = 0.027$ $P = 0.488$	$R^2 = 0.213$ $P = 0.072$
Nitrite (NO_2^-)	Total chlorine	$R^2 = 0.419$ $P = 0.007$	$R^2 = 0.081$ $P = 0.223$	$R^2 = 0.055$ $P = 0.380$
	Monochloramine /Free chlorine	$R^2 = 0.346$ $P = 0.013$	$R^2 = 0.103$ $P = 0.168$	$R^2 = 0.055$ $P = 0.380$
	Heterotrophic bacteria	$R^2 = 0.189$ $P = 0.094$	$R^2 = 0.014$ $P = 0.625$	$R^2 = 0.027$ $P = 0.545$

	NTM	R^2 =0.116 P = 0.197	R^2 = 0.052 P = 0.334	R^2 = 0.104 P = 0.224
Nitrate (NO_3^-)	Total chlorine	R^2 =0.021 P = 0.597	R^2 = 0.093 P = 0.192	R^2 = 0.045 P = 0.429
	Monochloramine /Free chlorine	R^2 = 0.009 P = 0.718	R^2 = 0.093 P = 0.192	R^2 = 0.029 P = 0.529
	Heterotrophic bacteria	R^2 = 0.048 P = 0.415	R^2 = 0.262 P = 0.021	R^2 = 0.051 P = 0.400
	NTM	R^2 = 0.000 P = 0.995	R^2 = 0.048 P = 0.352	R^2 = 0.006 P = 0.783
Grey boxes indicated significant correlation.				

3.4 Summary.

Supplemental Table S3: Water quality parameters median concentration and standard error for distribution samples, for all Phases.

Distribution only					
Water quality parameters	Phase 1 (P1)	Phase 2 (P2)	Phase 3 (P3)	P1 vs P3	P1 vs P2
Total chlorine (mg Cl_2/L)	2.3 ± 1.2	2.7 ± 0.8	1.9 ± 1.1	0.678	0.101
Monochloramine (mg Cl_2/L)	1.9 ± 1.1	0.0 ± 0.1	1.7 ± 1.1	0.734	<0.001
Free chlorine (mg Cl_2/L)	0.1 ± 0.1	2.7 ± 0.7	0.1 ± 0.1	0.936	<0.001
Heterotrophic bacteria (CFU/L)	(2 ± 12) × 10^4	5 × 10^3	(5 ± 36) × 10^4	0.177	<0.001
Nontuberculous mycobacteria (CFU/L)	10 ± 334	0.5	10 ± 530	0.762	0.001
Trihalomethane 4 (µg/L)	21.2 ± 7.2	63.0 ± 33.6	25.0 ± 7.5	0.646	0.108
Haloacetic acid 5 (µg/L)	12.4 ± 4.0	55.7 ± 32.3	17.5 ± 3.6	0.062	<0.001
Haloacetic acid 9 (µg/L)	16.6 ± 6.3	61.8 ± 32.3	25.7 ± 4.4	0.019	0.058
Free ammonia (mg NH_2-N /L)	0.22 ± 0.11	0.03 ± 0.10	0.18 ± 0.14	0.193	<0.001
Nitrate (mg/L)	0.48 ± 0.23	0.59 ± 0.13	0.41 ± 0.14	0.105	0.166
Nitrite (mg NO_2-N/L)	0.007 ± 0.026	0.007 ± 0.009	0.007 ± 0.002	0.788	0.987
pH	6.6 ± 0.2	6.6 ± 0.2	6.7 ± 0.1	0.011	0.854
Temperature (°C)	25.9 ± 1.6	27.6 ± 1.3	29.6 ± 1.6	<0.001	0.001
N/A = not assessed, **Location:** distribution sampling locations: entry point (EP), hydrant (DS-1), storage tank (STa) (STb), maximum residence time (MRT), homes: RG, RT, RW, RC.**: test type:** U or t-test, **significance:** $\alpha \leq 0.5$**, phase:** Phase 1 (P1), Phase 2 (P2), and Phase 3 (P3)					

Supplemental Table S4: Water quality parameters median and standard error concentrations for home samples for all Phases.

Home samples					
Water quality parameters	Phase 1 (P1)	Phase 2 (P2)	Phase 3 (P3)	P1 vs P3	P1 vs P2
Total chlorine (mg Cl_2/L)	2.0 ± 0.8	2.16 ± 0.6	1.7 ± 0.77	0.219	0.412
Monochloramine (mg Cl_2/L)	1.8 ± 0.7	0.0 ± 0.0	1.5 ± 0.6	0.257	<0.001
Free chlorine (mg Cl_2/L)	0.1 ± 0.0	2.0 ± 0.7	0.1 ± 0.0	0.438	<0.001
Heterotrophic bacteria (CFU/L)	$(3 \pm 4) \times 10^5$	$(5 \pm 17) \times 10^3$	$(4 \pm 8) \times 10^5$	0.782	0.001
Nontuberculous mycobacteria (CFU/L)	89 ± 407	2 ± 8	16 ± 505	0.463	<0.001
Trihalomethane 4 (µg/L)	N/A	N/A	N/A	N/A	N/A
Haloacetic acid 5 (µg/L)	N/A	N/A	N/A	N/A	N/A
Haloacetic acid 9 (µg/L)	N/A	N/A	N/A	N/A	N/A
Free ammonia (mg NH_2-N /L)	0.25 ± 0.09	0.03 ± 0.03	0.17 ± 0.1	0.040	<0.001
Nitrate (mg/L)	0.41 ± 0.18	0.54 ± 0.16	0.39 ± 0.13	0.260	0.006
Nitrite (mg NO_2-N/L)	0.007 ± 0.002	0.006 ± 0.001	0.007 ± 0.003	0.506	0.072
pH	6.8 ± 0.3	6.9 ± 0.1	6.8 ± 0.2	0.465	0.004
Temperature (°C)	24.8 ± 1.4	27.0 ± 1.6	26.4 ± 2.3	0.023	0.007
N/A = not assessed, **Location:** distribution sampling locations: entry point (EP), hydrant (DS-1), storage tank (STa) (STb), maximum residence time (MRT), homes: RG, RT, RW, RC.**: test type:** t-test, **significance:** $\alpha \leq 0.5$**, phase:** Phase 1 (P1), Phase 2 (P2), and Phase 3 (P3), N/A=not applicable					

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