Supporting Information

Effects of molecular ozone and hydroxyl radical on formation of *N*-Nitrosamines and perfluoroalkyl acids during ozonation of treated wastewaters

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1. SI Analytical Methods and Chemicals

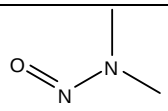
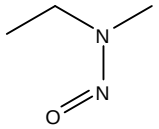
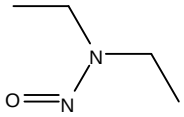
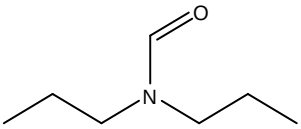
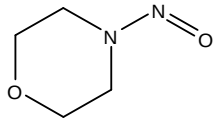
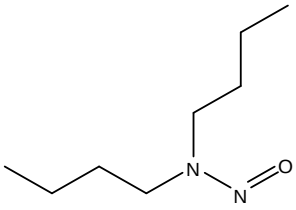
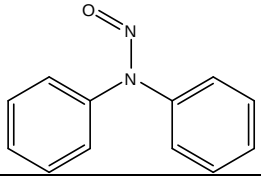
1.1 Chemicals

Trace analysis–grade methanol and dichloromethane (DCM) were obtained from Burdick and Jackson (Muskegon, MI). Sodium azide, mercuric chloride, 5 N sodium hydroxide, sodium dihydrogen phosphate, and disodium hydrogen phosphate were purchased from Fisher Scientific (Fair Lawn, NJ). Sodium thiosulfate and concentrated sulfuric acid were purchased from EM Science (Merck KGaA, Darnstadt, Germany). Reagent-grade water was prepared by using a Milli-Q Gradient water purification system (Millipore, Billerica, MA). Nitrosamine standards were purchased from Ultra Scientific (Kingstown, RI), and isotopically-labeled nitrosamines were purchased from Cambridge Isotope Laboratories (Andover, MA). PFAAs standards were purchased from Wellington Laboratories (Guelph, Ontario, Canada). Ascorbic acid was purchased from Mallinckrod Chemicals (Phillipsburg, NJ). Potassium indigotrisulfonate, iodine, sodium hypochlorite (10-14 wt% as free chlorine), ammonium chloride, and sulfanilimide were purchased from Sigma Aldrich (St. Louis, MO). Potassium monobasic phosphate, American Chemical Society grade, was purchased from Fisher (Thermo Fisher Scientific, Waltham, MA). Concentrated phosphoric acid was purchased from JT Baker (Avantor Performance Materials, Phillipsburg, NJ). Glacial acetic acid (HPLC grade) and ethylene glycol were purchased from EMD Chemicals, Inc. (Gibbstown, NJ). Potassium iodide (99%) was purchased from Alfa Aesar (Ward Hill, MA) and 99.5% pure tert-butanol was purchased from Acros Organics (Fairlawn, NJ). Sodium p-chlorobenzoate was purchased from Pfaltz & Bauer (Waterbury, CT) and concentrated hydrogen peroxide (34 wt%) was purchased from EnviroTech Chemical Services (Modesto, CA).

1.2 Analysis of *N*-Nitrosamines

Table SI-1 provides a summary of the nitrosamines included in this study. Samples were collected in 1 L precleaned, presilanized, amber glass bottles. Aliquots of sodium azide (1%) and sodium thiosulfate (0.8%) were added to bottles prior to sampling for preservation and to quench residual oxidant. After sampling, bottles were kept on ice during transportation to laboratory and stored at 4° C until extraction. All samples were extracted within 14 days of collection. When necessary, samples were filtered prior to extraction with 90 mm glass fiber (GF/F) filters. ASPE was performed using a Dionex AutoTrace workstation (Thermo Scientific, Sunnyvale, CA).

Table SI-1 Nitrosamines, CAS Numbers, Structures, and Corresponding Isotopes

Compound	CAS#	Structure	Isotope
N-Nitrosodimethylamine (NDMA)	62-75-9		NDMA-d_6
N-Nitrosomethyl-ethylamine (NMEA)	10595-95-6		NMEA-d_3
N-Nitrosodiethylamine (NDEA)	55-18-5		NDEA-d_{10}
N-Nitrosodipropylamine (NDPA)	621-64-7		NDPA-d_{14}
N-Nitrosomorpholine (NMOR)	59-89-2		NMOR-d_8
N-Nitrosodibutylamine (NDBA)	924-16-3		NDBA-d_{18}
N-Nitrosodiphenylamine (NDPh)	86-30-6		NDPh-d_6

Note: CAS=Chemical Abstract Services

Samples (1 L) were processed in batches of six and spiked with 100 µL of isotope mix at 0.5 to 2.5 mg/L for a concentration of 100 to 500 µg/L in the final extract. Prepacked activated charcoal cartridges (Resprep 521, Restek, Bellefonte, PA) were sequentially conditioned with 5 mL DCM, 5 mL methanol, and 10 mL reagent-grade water with a flow rate of 15 mL/min. Samples were loaded at a rate of 15 mL/min. Cartridges were rinsed with 5 mL reagent-grade water with a flow rate of 20 mL/min and dried for 10 min with nitrogen gas. Analytes were eluted with 10 mL DCM into 15 mL conical vials (Dionex) with a flow rate of 5 mL/min. Extracts were evaporated under nitrogen gas to approximately 2 mL. Water was then removed from the DCM extracts by passing the 2 mL extract through a DryDisk separation membrane (Horizon Technology, Salem, NH). The DCM extract was collected and concentrated to a final volume of 500 µL with nitrogen gas, resulting in a 1:2000 concentration factor. A Varian

(Walnut Creek, CA) CP-3800 gas chromatograph with a CP-8400 auto sampler was used for all analyses. The injector (Varian 1177) was operated in splitless mode with a Siltek™ deactivated glass liner (Restek, Bellefonte, PA) and set at 200 °C. Analytes were separated on a 30 m x 0.32 mm ID x 1.4 µm DB624 column (J & W, Agilent, Palo Alto) using a 1.4 mL/min helium flow with an initial pressure pulse of 35 psi for 0.85 min. The temperature program was as follows: 35 °C, hold for 1.0 min; 35 to 120 °C at 5 °C/min; 120 to 145 °C at 3 °C/min; 145 to 250 °C at 35 °C/min, hold for 4.64 min. An injection volume of 2 µL was used for all analyses. The transfer line was set at 240 °C. Analysis was performed using a Varian 4000 ion trap mass spectrometer (Walnut Creek, CA). All analyses were performed using multiple reaction monitoring in positive chemical ionization mode using liquid methanol. *N*-nitrosopiperidine and *N*-nitrosopyrrolidine were initially included in the analysis but were removed because matrix interference resulted in unreliable quantification. Precursor and product ions used for quantitation and confirmation are listed in Table SI-2 for target nitrosamines as well as their molecular weights and method reporting limits (MRL). Some of the nitrosamines did not exhibit a second product ion in high enough abundance to monitor as a confirmation transition and therefore only have one quantitation transition. Because of thermal degradation upon injection, *N*-nitrosodiphenylamine was analyzed as diphenylamine. Quantitation was performed using isotope dilution. Method reporting limits were established at 3 to 5 times the calculated method detection limit (MDL; n=12). A minimum of seven calibration standards were used to construct a calibration curve for each analyte, with at least one calibration standard analyzed at or below the MRL. Correlation coefficients were required to be at least 0.990 but typically exceeded 0.995 using linear regression. A field blank was collected for each sampling event, extracted, and analyzed. A laboratory reagent blank was also included in each extract batch. Acceptance criteria for a data batch required any observable compound peaks in blanks to remain at less than 1/3 MRL; otherwise, results were flagged, and compound MRL was adjusted for all samples in batch. Laboratory fortified reagent blanks and sample matrices and a sample duplicate were incorporated into each extract batch to monitor analytical performance. Acceptance limits for recovery were set at 70 to 130% and at 30% relative difference for duplicates. Table SI-3 displays the average analytical error for replicate analysis of each compound and recovery summaries for reagent water and matrix spikes.

Table SI-2 Molecular Weights, MRM Transition Ions, and MRLs

Compound	MW (amu)	Precursor ion (m/z)	Product ion (m/z)	MRL (ng/L)
NDMA	74	75	47 (44, 43, 58) [b]	2.5
NMEA	88	89	61 (47)	2.5
NDEA	102	103	75	5.0
NDPA	130	131	89	10
NMOR	116	117	86 (87)	5.0
NDBA	158	159	103	10
NDPhA	198 (169)[a]	170	92 (143)	10

Notes: [a]=analyzed as diphenylamine; [b]=( )-confirmation product ions; MRL=method reporting limit; MRM=multiple reaction monitoring.

Table SI-3 Average Recovery and Relative Standard Deviations for Target Nitrosamines in Various Water Matrices (n=6) Spiked at 25 ng/L

	Reagent Water		Finished Drinking Water		Surface Water		Tertiary Wastewater	
	Average %	RSD %	Average %	RSD %	Average %	RSD %	Average %	RSD %
NDMA	114	4.0	117	3.2	117	0.89	136	2.1
NMEA	99	3.1	98	1.5	101	2.1	99	2.8
NDEA	98	6.2	104	6.2	97	5.0	101	5.7
NDPA	109	10	82	9.9	105	7.3	78	10
NMOR	107	7.2	100	6.8	101	4.6	109	9.8
NDBA	105	6.8	98	9.1	95	7.7	47	5.7
NDPhA	84	6.1	87	5.9	89	2.8	105	4.9

Notes: RSD=relative standard definitions.

1.3 Analysis of Perfluoroalkyl Acids

Analytical standards and isotopically-labeled standards for all PFAAs measured in this study (Table SI-4) were procured from Wellington Laboratories (Guelph, Ontario, Canada. Whenever possible, matched isotope standards were used for quantitation of each PFAA. Working stock PFAA solutions and calibration standards were prepared in methanol, and appropriate dilutions were made for ASPE spiking solutions. All solutions and standards were stored at -20° C.

All samples shipped to the Southern Nevada Water Authority (SNWA) laboratory were collected in 1 L precleaned, wide mouth, amber high density polyethylene bottles (Rochester, NY). An aliquot of ascorbic acid solution (0.05%) was added to all bottles prior to sampling for chlorine quenching. After sampling, bottles were kept on ice during transportation and stored at 4° C until extraction. Samples were extracted within 14 days of collection and, when necessary, filtered prior to extraction with prewashed 90 mm glass fiber filters. Preliminary studies indicated no impact from filtration on the measured concentrations of target analytes.

ASPE was performed using Dionex AutoTrace 280 workstation (Thermo Scientific, Sunnyvale, CA). Samples (1 L) were acidified to a pH greater than 2 with concentrated sulfuric acid, then spiked with isotopically labeled standards prior to extraction. Samples were processed in batches of six. Prepacked 200 mg, 6 cc Hydrophilic-Lipophilic-Balanced (HLB) cartridges (Waters Corporation, Milford, MA) were sequentially conditioned with 5 mL MTBE, 5 mL methanol, and 5 mL reagent water at a flow rate of 15 mL/min. Samples were loaded at a rate of 15 mL/min. Cartridges were rinsed with 5 mL reagent water and dried for 30 min with nitrogen gas. Target analytes were eluted with 10 mL of methanol into 15 mL conical vials (Dionex) at a flow rate of 5 mL/min. Extracts were concentrated to a final volume of 500 µL or 1 mL with nitrogen gas.

Table SI-4 Suite of Measured PFAAs in This Study

PFAA Classes	Chemical Name	Abbreviation	CAS RN	M.W. (g/mol)	Molecular Formula	Relevant Guidance Levels
Perfluoro-carboxylic acids (PFCAs)	Perfluorobutyric acid	PFBA	375-22-4	214	$C_4HF_7O_2$	7.0 µg/L[b]
	Perfluoropentanoic acid	PFPnA	2706-90-3	264	$C_5HF_9O_2$	
	Perfluorohexanoic acid	PFHxA	307-24-4	314	$C_6HF_{11}O_2$	
	Perfluoroheptanoic acid	PFHpA	375-85-9	364	$C_7HF_{13}O_2$	
	Perfluorooctanoic acid	PFOA	335-67-1	414	$C_8HF_{15}O_2$	0.4 µg/L[a], 0.3 µg/L[b], 0.04 µg/L[c]
Perfluoro-sulfonic acids (PFSAs)	Perfluorobutane sulfonic acid	PFBS	375-73-5	300	$C_4HF_9SO_3$	7.0 µg/L[b]
	Perfluorohexane sulfonic acid	PFHxS	355-46-4	400	$C_6HF_{13}SO_3$	
	Perfluorooctane sulfonic acid	PFOS	1763-23-1	500	$C_8HF_{17}SO_3$	0.2 µg/L[a], 0.3 µg/L[b]

Notes: [a]=U.S. Environmental Protection Agency PHA values; [b]=Minnesota Department of Health Health Risk Limits; [c]=New Jersey Department of Environmental Protection health-based drinking water guidance level; CAS RN=Chemical Abstract Services Registry Number; MW=molecular weight; PFAA=perfluoroalkyl acids.

Analysis of ASPE extracts was conducted at SNWA's research and development laboratory via liquid chromatography–tandem mass spectrometry (LC–MS/MS). Briefly, an Agilent (Palo Alto, CA) G1312A binary pump and an HTC-PAL auto sampler (CTC Analytics, Zwingen, Switzerland) were used. Analytes were separated using a 150 Å 4.6 mm Synergi Max-RP C12 column with a 4 µm pore size (Phenomenex, Torrance, CA) and a binary gradient consisting of 5.0 mM ammonium acetate (v/v) in water (A) and 100% methanol (B) at a flow rate of 800 µL/min. An injection volume of 10 µL was used for all analyses. Contaminants from the aqueous channel were removed using a 4.0 x 10 mm Hypercarb (Thermo Fisher Scientific, Waltham, MA) drop-in guard cartridge attached in-line before the instrument's purge valve. Remaining contaminants were separated from analyte peaks by installing a 75 x 4.6 mm Synergy Max-RPC12 column with a 4 µm pore size (Phenomenex, Torrance, CA) in-line upstream from the injector valve. Tandem mass spectrometry was performed using an API 4000 triple quadrupole mass spectrometer (AB SCIEX, Foster City, CA). Using electrospray ionization (ESI) operated in negative ionization mode, optimal compound-dependent parameters were determined for additional analytes, and source-dependent parameters were optimized. The concentration of each analyte was determined by isotope dilution, surrogate standard, or external calibration. MRLs were based on MDLs calculated from seven replicate measurements of deionized water samples fortified with analytes and extracted as previously described. As an added cautionary measure, MRLs for each analyte were set conservatively at least five times the MDL, higher as needed in consideration of known and unanticipated background sources. Compound-dependent analytical and quantitation parameters are detailed in Table SI-5.

A minimum of seven calibration standards were used to construct a calibration curve for each analyte, with at least one calibration standard analyzed at or below the MRL. Correlation coefficients were required to be at least 0.990 but typically exceeded 0.995 using linear regression. A field blank was collected for each sampling event, extracted, and analyzed. A

laboratory reagent blank was also included in each extract batch. Acceptance criteria for a data batch required that any observable compound peaks in blanks remain at less than 1/3 MRL; otherwise, results were flagged, and compound MRL was adjusted for all samples in the batch. Laboratory fortified reagent blanks and sample matrices and a sample duplicate were incorporated into each extract batch to monitor analytical performance. Acceptance limits for recovery were set at 70 to 130% and at 30% relative difference for duplicates. Signal counts for internal and surrogate standard peaks were required to remain higher than 10% when compared to average peak counts in calibrators. Samples not meeting these criteria were reanalyzed and diluted for matrix reduction as needed. Samples where efforts did not produce acceptable quality control criteria were flagged as such. Table SI-6 displays the average analytical error for duplicate analysis of each compound and recovery summaries for reagent water and matrix spikes for the project.

Table SI-5 Compound-Dependent Analytical and Quantitation Parameters

Abbreviation	Retention Time (min)	MRM[a] Transition	Quantitation	Calibration Range (µg/L)	Method Reporting Limit (ng/L)
PFBA	6.3	213>169	isotope dilution ([$^{13}c_4$] pfba)	0.50–125	5
PFPnA	7.1	263>219	isotope dilution ([$^{13}c_5$] pfpna)	0.50–125	2
PFHxA	8.2	313>269	isotope dilution ([$^{13}c_2$] pfhxa)	0.10–25	0.5
PFHpA	9.4	363>319	isotope dilution ([$^{13}c_4$] pfhpa)	0.10–25	0.5
PFOA	10.2	413>369	isotope dilution ([$^{13}c_4$] pfoa)	0.50–125	5
PFBS	7.1	299>99	surrogate standard ([$^{18}o_2$] pfhxs)	0.10–25	0.25
PFHxS	9.4	399>80	isotope dilution ([$^{18}o_2$] pfhxs)	0.10–25	0.25
PFOS	10.7	499>80	isotope dilution ([$^{13}c_4$] pfos)	0.10–25	0.25

Notes: MRM=multiple reaction monitoring.

Table SI-6 Analytical Variability and Spike Recovery Data

	Variability of Replicate Samples		Spike Recoveries in Reagent Water (n=49)			Matrix Spike Recoveries (n=12)		
Abbreviation	Average % difference (n=12)	Max	Spike Conc. (ng/L)	Mean Recovery (%)	RSD (%)	Spike Conc. (ng/L)	Mean Recovery (%)	RSD (%)
PFBA	2	3	20	102	6.2	20	105	13.5
PFPnA	4	9	20	107	12.3	20	110	9.3
PFHxA	4	14	10	105	12.2	10	109	12.0
PFHpA	7	24	10	110	14.1	10	114	16.6
PFOA	9	19	20	100	14.3	20	96	13.4
PFBS	8	25	10	112	10.0	10	114	8.7
PFHxS	5	13	10	98	11.6	10	88	17.4
PFOS	9	20	10	104	10.4	10	114	15.9

Notes: RSD=relative standard deviations.

1.4 Effluent Organic Matter (EfOM) Characterization TOC

Effluent organic matter in the wastewater samples was characterized using organic carbon analysis, collection of fluorescence excitation-emission spectra, and absorbance measurement. For dissolved organic carbon (DOC) and total organic carbon (TOC) analyses, samples were collected into glass vials, acidified to a pH less than 3 with hydrochloric acid. When needed, samples were filtered through a 0.45 µm hydrophilic polypropylene filter (GHP Acrodisk, Pall Life Sciences) and reported as DOC. A Shimadzu (Shimadzu Scientific Instruments, Carlsbad, CA) TOC/total nitrogen analyzer was used for quantification.

Excitation–emission matrices (EEMs) were analyzed by the use of a fluorescence regional integration (FRI) method, which was modified and described previously.[18-20] The EEM integration was based on three regions, consisting of a microbial byproducts-biopolymer region, fulvic-like substances, and humic-like substances. To avoid any bias from excitation wavelength (E(λ)), a boundary for the integration regions at [E(λ)-15 nm] was used. Similarly, to avoid any bias from the second order of the excitation wavelength, an upper boundary of [2 x E(λ) – 15 nm] was used. Sample absorbance at 254 nm was measured using a Perkin-Elmer Lambda 45 UV-VIS spectrometer, consistent with Standard Method 5910 B. Specific UV_{254} absorbance (SUVA; $L \cdot m^{-1} \cdot mg^{-1}$) was calculated by dividing TOC concentration by absorbance at 254 nm.

2. Results

2.1 Water Quality of tested wastewaters

Corresponding water quality parameters for the six US wastewaters included in this study are shown in Table SI-7. Corresponding fluorescence excitation-emission matrices shown in Supporting Information Figure SI-1.

Table SI-7. Water Quality Parameters for Wastewaters in this Study

Parameter	Unit	WW1	WW2	WW3	WW4	WW5	WW6
COD	mg/L	54	<20	N/A	N/A	N/A	N/A
BOD	mg/L	<2	<2	6.0	N/A	7.2	N/A
Total P	mg/L	0.30	0.12	0.2	N/A	6.0	N/A
NH_4^+	mg-N/L	12.5	3.53	0.26	N/A	0.2	N/A
TKN	mg-N/L	16.0	4.2	<1.0	N/A	N/A	N/A
NO_3^-	mg-N/L	0.3	14.1	14.5	<1.0	N/A	N/A
Total nitrogen	mg-N/L	16.3	18.3	14.5	57	6.1	7.6
UV_{254}	a.u.	0.12	0.09	0.12	0.24	0.35	0.09
TOC	mg-C/L	6.1	4.5	5.1	14	17	4.1
SUVA	L/mg-m	2.09	1.92	2.27	N/A	N/A	N/A
TF	a.u	36,721	23,530	34,050	55,451	253,639	23,016
FI	a.u	1.55	1.62	1.73	1.53	1.03	1.47
TDS	mg/L	870	N/A	980	N/A	N/A	N/A

Notes: BOD=biological oxygen demand; COD=chemical oxygen demand; FI=fluorescence index; N/A=not available; P=phosphorus; SUVA=specific ultraviolet absorbance; TKN=total Kjeldahl nitrogen; TDS=total dissolved solids; TF=total integrated fluorescence; TOC=total organic carbon

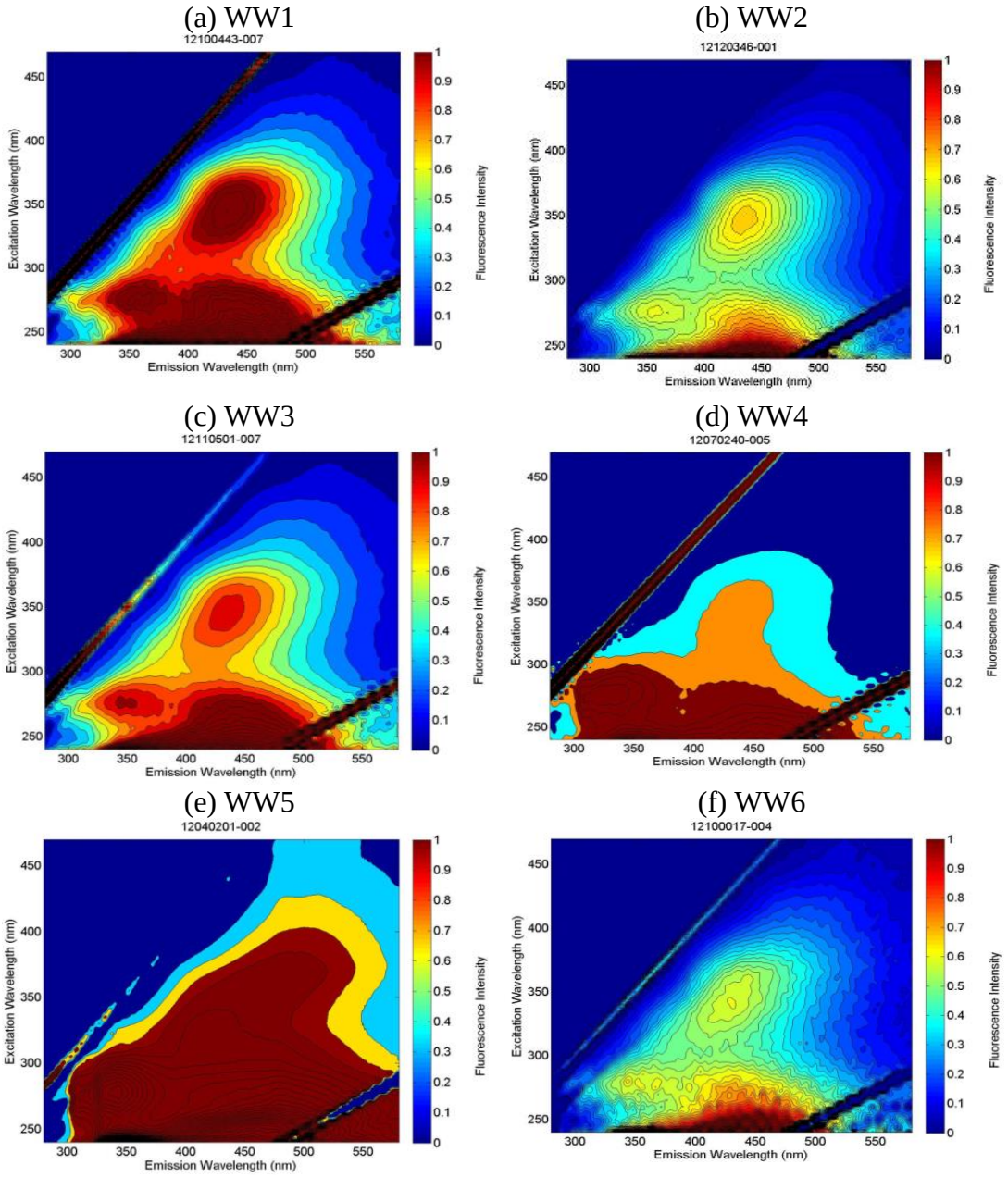


Figure SI-1. Excitation-Emission Matrices of (a) WW1; (b) WW2; (c) WW3; (d) WW4; (e) WW5; (f) WW6.

2.1 Effects of O_3 and O_3/H_2O_2 dose conditions on *N*-nitrosamines

Table SI-8 provides a summary of formation of the other nitrosamines.

Table SI-8. Changes in NMEA, NDEA, and NMOR After Ozonation

	WW1	WW5	WW3	WW4	WW6
O_3:TOC Ratio	NMEA ng/L	NDEA ng/L	NMOR ng/L	NMOR ng/L	NMOR ng/L
(no ozone)	<5.5	<14	12	<12	17
0.10	<5.5	<14	N/A	<12	N/A
0.20	<5.5	N/A	12	<12	16
0.30	6.0	N/A	N/A	12	N/A
0.40	N/A	N/A	N/A	<12	N/A
0.50	N/A	N/A	N/A	<12	14
0.75	N/A	N/A	N/A	N/A	14
1.0	7.4	18	<11	13	13
1.0/H_2O_2	7.9	17	<11	N/A	16

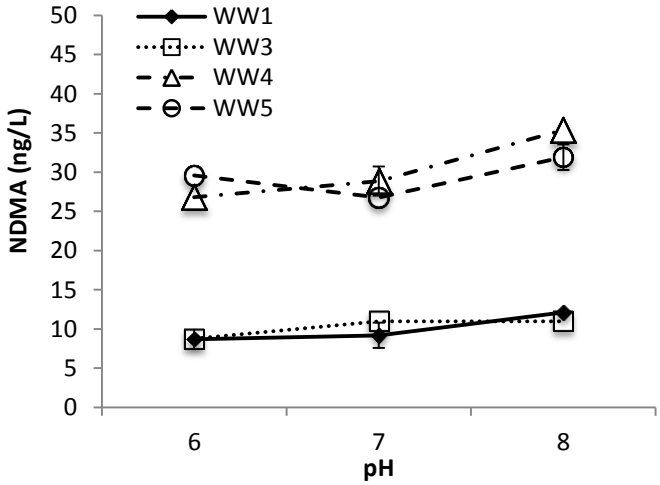


Figure SI-2. Formation of NDMA at various initial pH at a constant O_3:TOC ratio of 0.20.
Note: Error bars represent ± 1 standard deviation based on n=3 replicates.

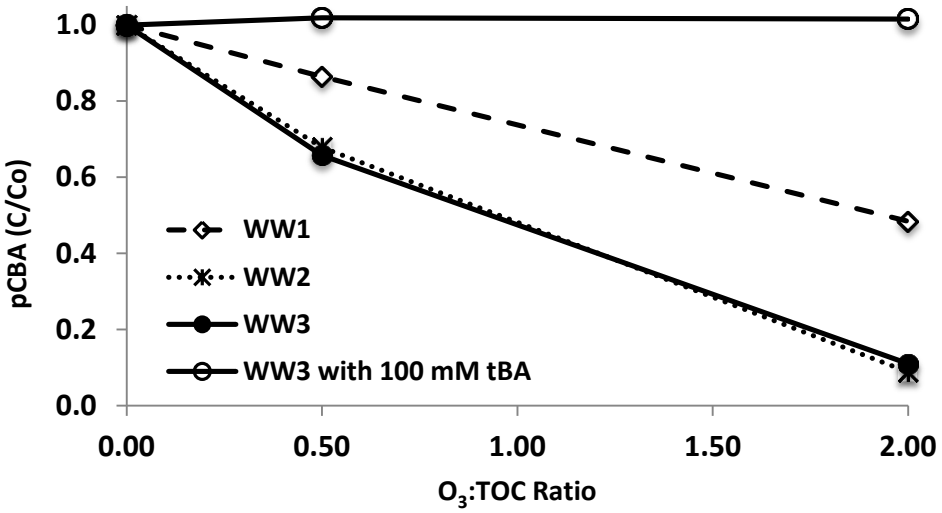


Figure SI-3. Decomposition of pCBA in WW1, WW2, and WW3 at various O_3:TOC ratios during bench-scale ozonation experiments.

2.3 Effects of O_3 and O_3/H_2O_2 dose conditions on PFAAs

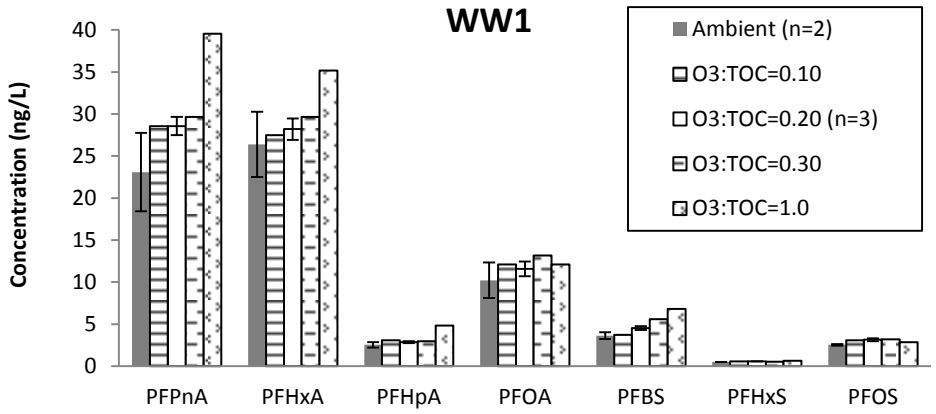


Figure SI-4. Change in PFAA in WW1—MBR filtrate (partial nitrifcation) at various O_3:TOC ratios.

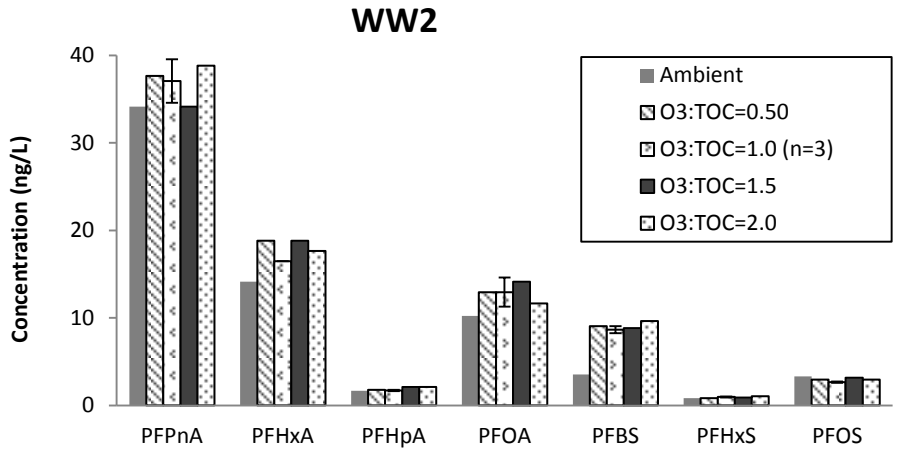


Figure SI-5. Change in PFAA in WW2—secondary effluent (nitrification) at various O_3:TOC ratios.

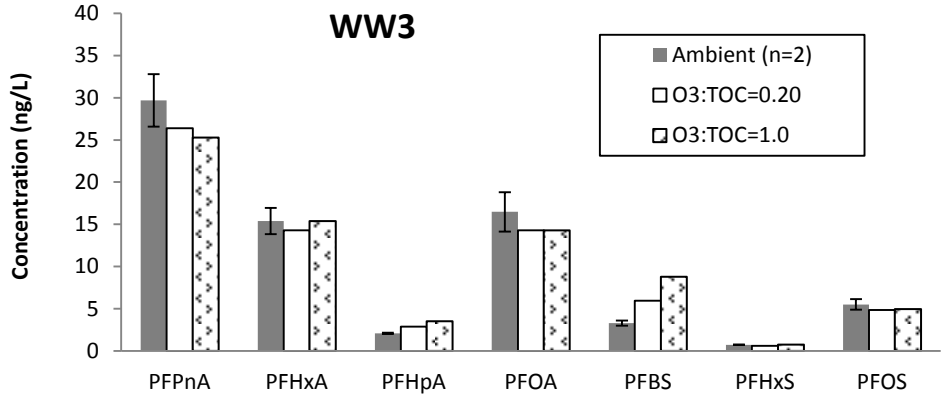


Figure SI-6. Change in PFAA in WW3—secondary effluent (nitrification/denitrification) at various O_3:TOC ratios.

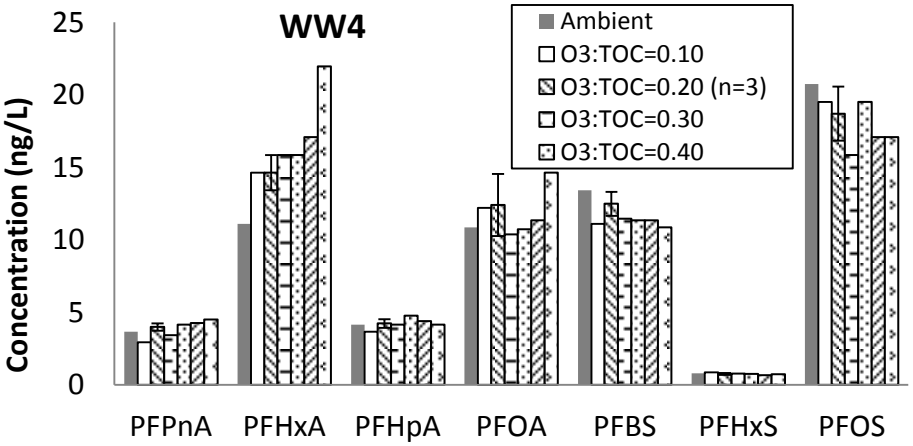


Figure SI-7. Change in PFAA in WW4—secondary effluent (BOD removal) at various O_3:TOC ratios.

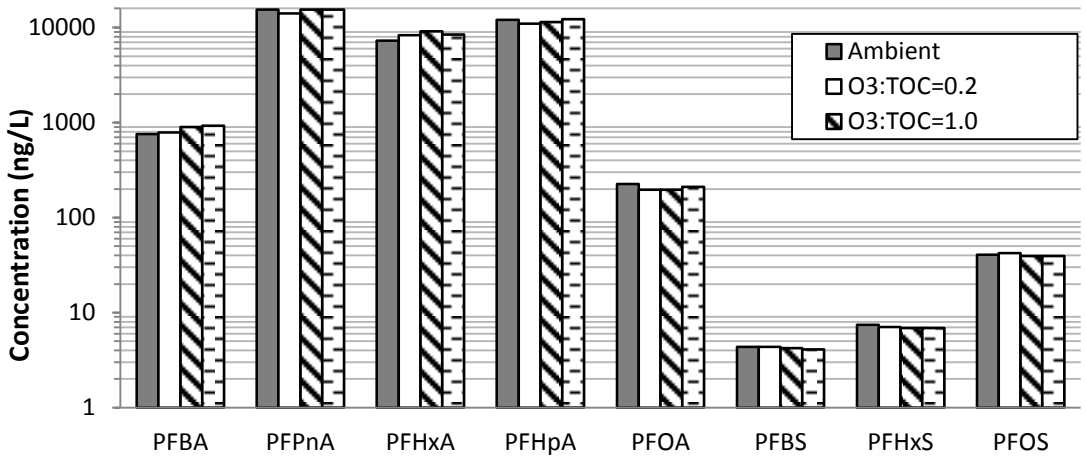


Figure SI-8. Change in PFAA in WW5—secondary effluent (BOD removal) at various O_3:TOC ratios. *Note*: Logscale y-axis

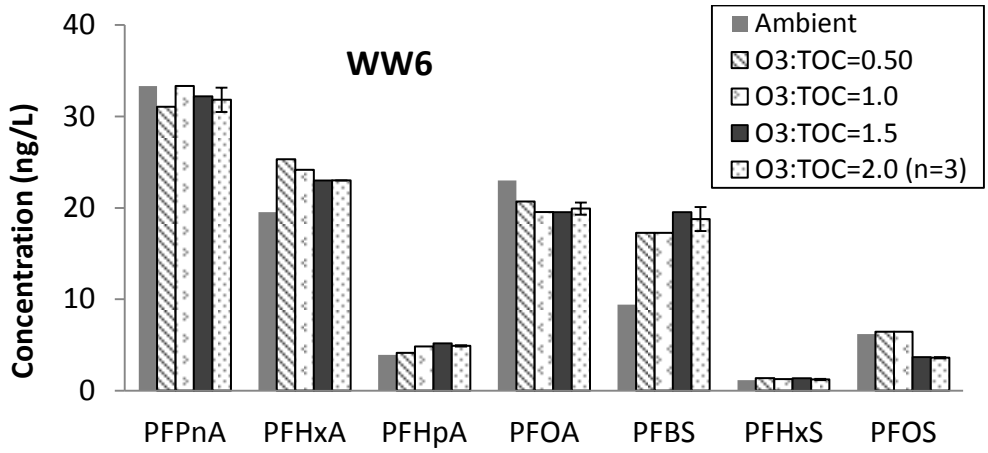


Figure SI-9. Change in PFAA in WW6—secondary effluent (nitrifying/denitrifying) at various O_3:TOC ratios.

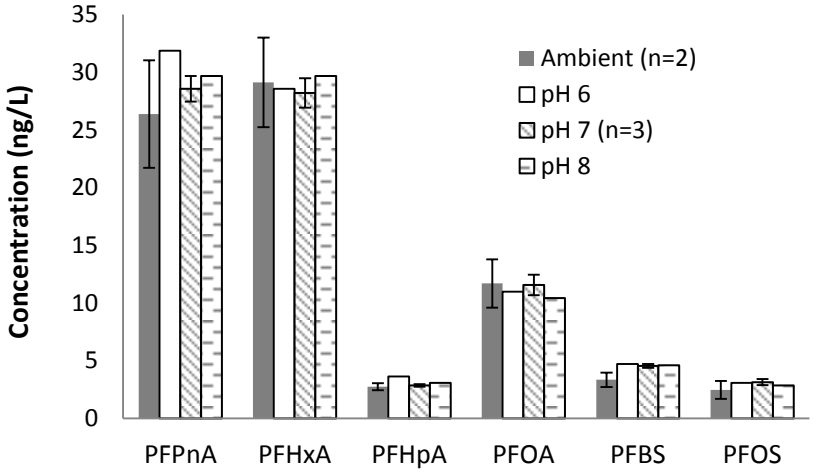


Figure SI-10. Change in PFAA formation in WW1 at various pH and O_3:TOC=0.20.

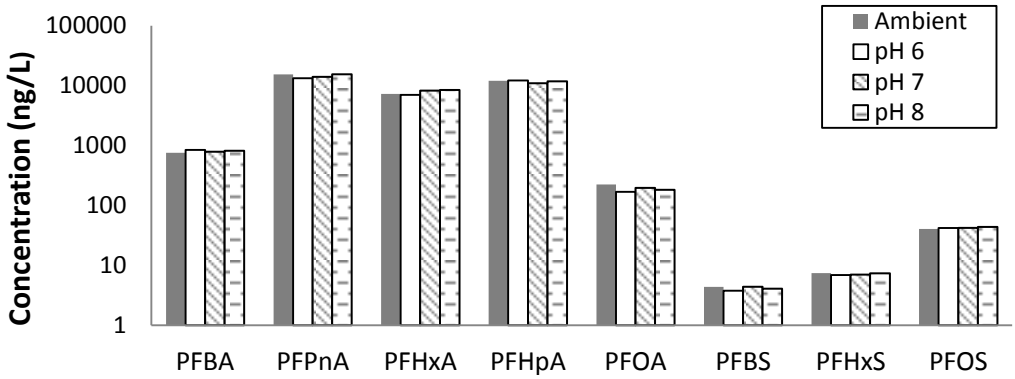


Figure SI-11. Change in PFAA formation in WW5 at various pH and O_3:TOC=0.20. *Note*: Logscale y-axis.

2.4 Effects of O_3 and •OH on PFAAs

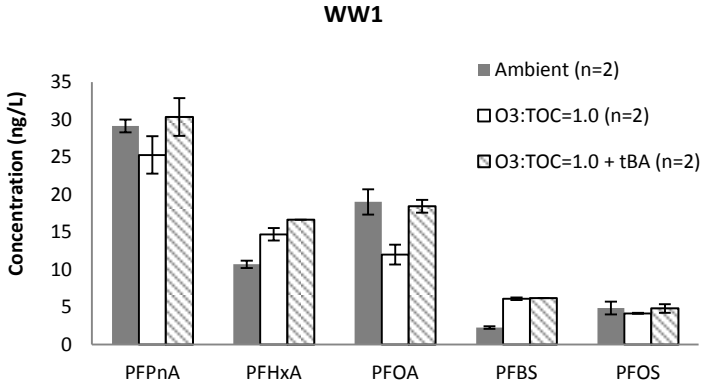


Figure SI-12. PFAA formation for WW1 after ozonation with and without tBA addition at O_3:TOC=1.0.

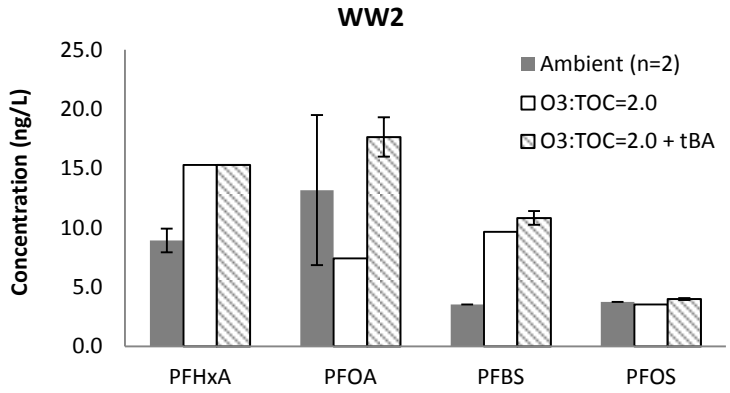


Figure SI-13. PFAA formation for WW2 after ozonation with and without tBA addition at O_3:TOC=2.0.

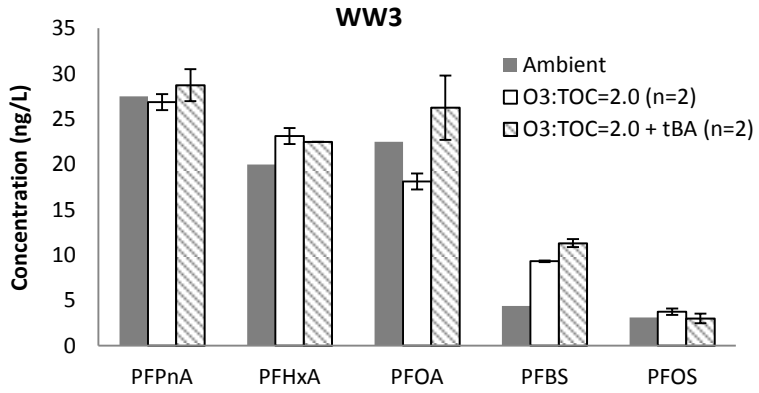


Figure SI-14. PFAA formation for WW3 after ozonation with and without tBA addition at O_3:TOC=2.0.

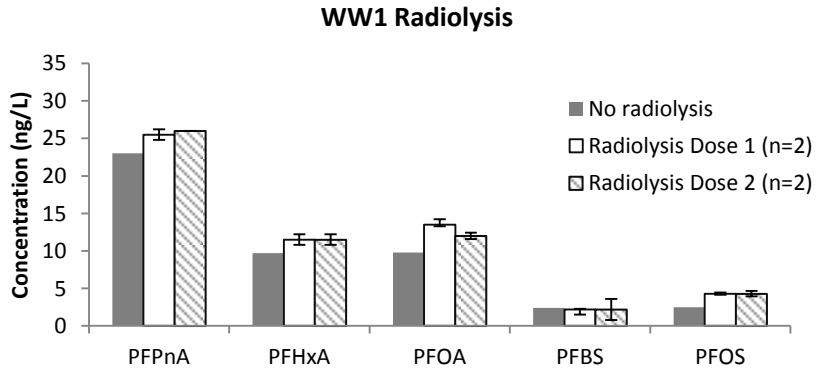


Figure SI-15. PFAA formation for WW1 before and after radiolysis.

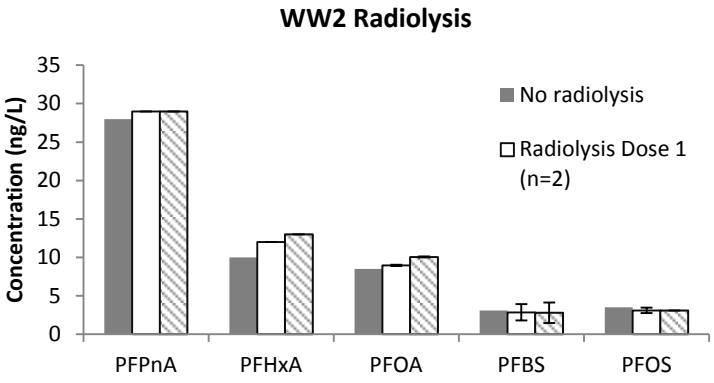


Figure SI-16. PFAA formation for WW2 before and after radiolysis.

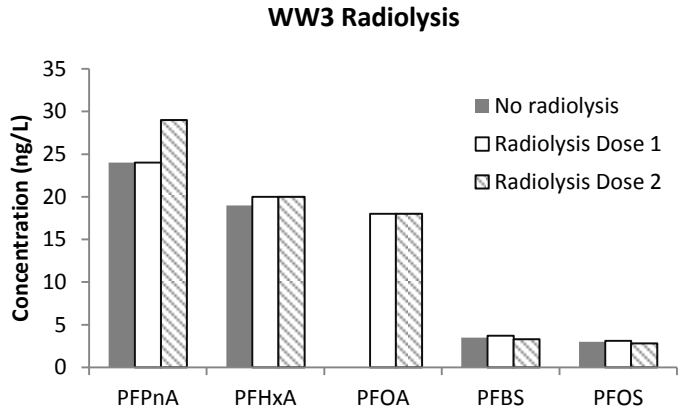


Figure SI-17. PFAA formation for WW3 before and after radiolysis.