Chlorination and Bromination of Nucleobases: The Role of Cytosine and Adenine in the Formation of Disinfection Byproducts

Julia E. Stroud[1], Kumudu H. Rathnayake[1], Susana Y. Kimura[1*]

[1]*Department of Chemistry, University of Calgary, 2500 University Dr. NW, Calgary, AB T2N 1N4, Canada*

[*]Corresponding author

E-mail address: s.kimurahara@ucalgary.ca

Table S1. Optimized parameters for all DBPs used in this study, including chemical transitions, dwell times, and collision energies

DBP Class	DBP	Purity (%)	Abb.	Retention time (min)	MDLs (ng/L)	Precursor	Quantification ion		Qualification ion		Dwell time
						(m/z)	m/z	CE (eV)	m/z	CE (eV)	(ms)
THM	Trichloromethane	N/A	TCM	2.46	4.8	83	48	40	47	30	3.5
	Tribromomethane	N/A	TBM	6.68	4.3	173	91.9	55	93.9	55	9.7
	Bromodichloromethane	N/A	BDCM	3.04	3.7	83	48	40	47	30	4.8
	Dibromochloromethane	N/A	DBCM	4.4	6.2	128.8	48	45	50	45	5.5
HAN	Chloroacetonitrile	99.5[b]	CAN	5.08	5.7	75	48	5	40.1	15	21.1
	Bromoacetonitrile	99.8[b]	BAN	7.55	3.6	120.9	40.1	10	41.1	10	18.3
	Iodoacetonitrile	98.1[b]	IAN	10.3	6.3	166.9	40.1	21	41.1	42	12.5
	Dichloroacetonitrile	99.4[d]	DCAN	4.74	3.2	73.9	47	21	40.1	32	22.2
	Dibromoacetonitrile	95.9[d]	DBAN	9.61	68.9	117.9	90.9	21	40.1	35	14.6
	Bromochloroacetonitrile	95.8[d]	BCAN	7.36	3.7	73.9	47	21	40.1	32	18.3
	Trichloroacetonitrile	98.0[d]	TCAN	3.3	3.2	107.8	72.9	29	47	60	21.7
HNM	Dichloronitromethane	96.2[a]	DCNM	5.81	4.1	82.9	48	52	47	55	27.8
HAL	Trichloroacetaldehyde	94.2[a]	TCAld	4.03	4.3	82	80.8	35	47	40	5.1
	Dibromochloroacetaldehyde	90.3[a]	DBCAld	8.69	11.9	128.9	48	48	47	50	18.8
	Tribromoacetaldehyde	97.3[b]	TBAld	10.6	13.0	172.8	91.9	59	93.9	58	20.8
HKT	1,1-dichloropropanone	95.5[d]	11DCP	5.63	25.7	82.9	47	43	48	43	23.3
	1,3-dichloropropanone	99.9[a]	13DCP	10.41	6.8	77	49	9	48	43	12.5
	1,1,1-trichloropropanone	98.7[d]	111TCP	8.42	30.6	124.9	97	9	82.9	9	18.8
	1-bromo-1,1-dichloropropanone	96.0[c]	1B11DCP	10.3	56.2	124.9	97	2	43.1	22	20.8
	1,1,3,3-Tetrachloropropanone	92.7[a]	1133TeCP	12.32	5.5	82.9	47	43	48	34	33.3
ITHM	Dichloroiodomethane	99.9[a]	DCIM	5.01	5.7	209.9	82.9	1	84.9	12	22.2

	Bromochloroiodomethane	99.0[a]	BCIM	7.29	7.5	255.9	128.8	2	130.8	11	15.4
	Dibromoiodomethane	93.9[a]	DBIM	9.27	2.0	172.8	91.9	57	93.9	57	18.8
	Chlorodiiodomethane	99.9[a]	CDIM	9.73	3.6	174.9	48	53	47	60	20.8
	Iodoform	99.0[b]	TIM	13.3	3.1	266.8	140	60	127	60	50.0
I.S	1,2-dibromopropane	97.0[b]	I.S.	7.2	N/A	120.9	92.9	30	41.1	10	16.3

[a]CanSyn Chem Corp [b]Sigma Aldrich [c]Toronto Research Chemicals [d]AccuStandard, I.S.: internal standard; N/A: not applicable; MDLs: method detection limits; Abb.: abbreviation. THM: trihalomethane; HAN: haloacetonitrile; HNM: halonitromethane; HAL: haloacetaldehyde; HKT: haloketone; ITHM: iodo-trihalomethane

Tables S2-S30 in downloadable excel file (.xlsx) for ease of access.

Text S1. Sample preparation for DBP quantification

Individual DBP reference standards were weighed and dissolved in anhydrous ACN to produce ~4000 mg/L stock solutions. Six 100 mg/L class-specific DBP stock solutions were prepared by combining individual components in anhydrous ACN. Both individual and mixed DBP stock solutions remained stable for up to one year. Two master stock solutions (100 and 5 μg/L) were prepared daily by combining the DBP class-specific solutions. These master stocks were used to prepare calibration standards and to spike CDFW.

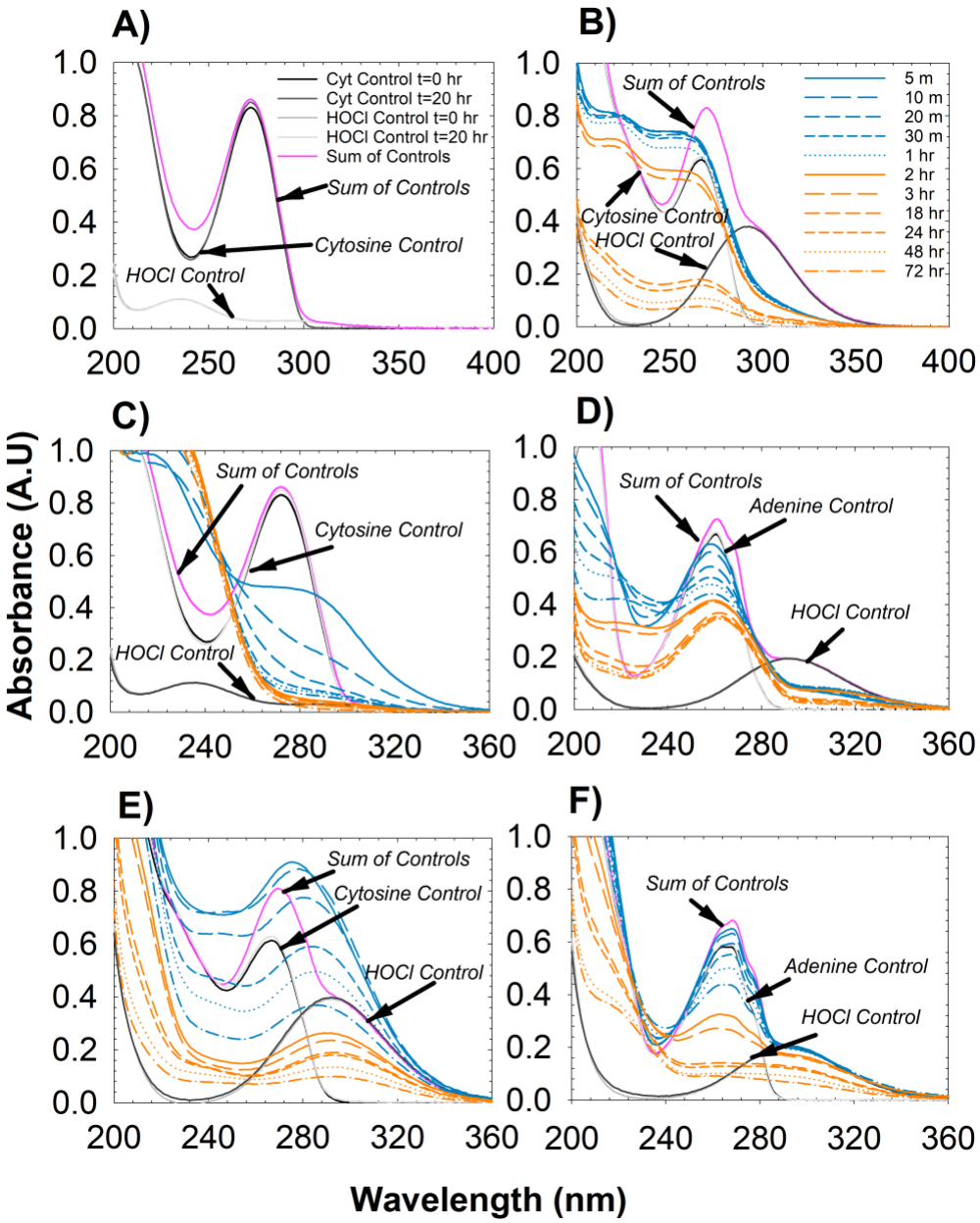

Figure S1. (A) depicts the controls isolated from (C) (0.1 mM Cytosine, 1 mM NaOCl, 5 mM acetate buffer), showing the lack of change in control concentration over time (t = 0, 20 hours) due to presence of acetate buffer under these reaction conditions. Reaction progress monitoring using UV-Visible spectrophotometry is shown for the reaction at pH7 of Cl/Cyt=10 (0.1 mM cytosine, 1mM NaOCl, 5 mM phosphate buffer) (B); as well as Cl/Cyt=10, pH4 (0.1 mM cytosine, 1 mM NaOCl, 5 mM acetate buffer) (C); Cl/Aden=10, pH7 (0.05 mM adenine, 0.5 mM

NaOCl, 5 mM phosphate buffer) (D); Cl/Cyt=10, pH10 (0.1 mM cytosine, 1 mM NaOCl, 5 mM carbonate buffer) (E); and Cl/Aden=10, pH10 (0.1 mM adenine, 1 mM NaOCl, 5 mM carbonate buffer) (F).

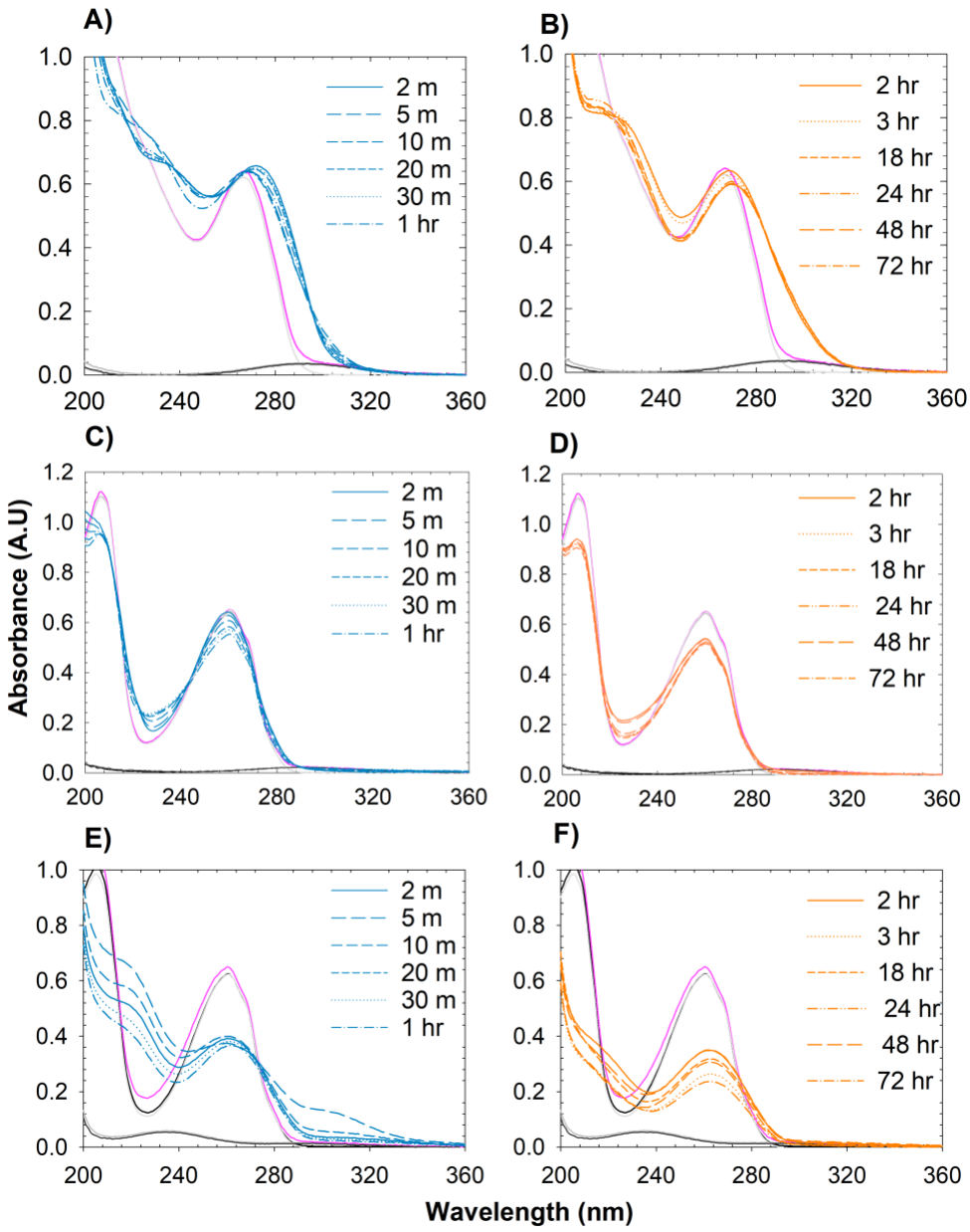

Figure S2. Reaction monitoring by UV-Visible spectrophotometry split by time points to improve visualization. The reaction progress is shown for: Cl/Cyt = 1, pH 7 t = 2 min to 1 hour (A), Cl/Cyt = 1, pH 7 t = 2 to 72 hours (B), Cl/Aden = 1, pH 7 t = 2 min to 1 hour (C), Cl/Aden = 1, pH 7 t = 2 to 72 hours (D), Cl/Aden = 10, pH 4 t = 2 min to 1 hour (E), and Cl/Aden = 10, pH 4 t = 2 to 72 hours (F).

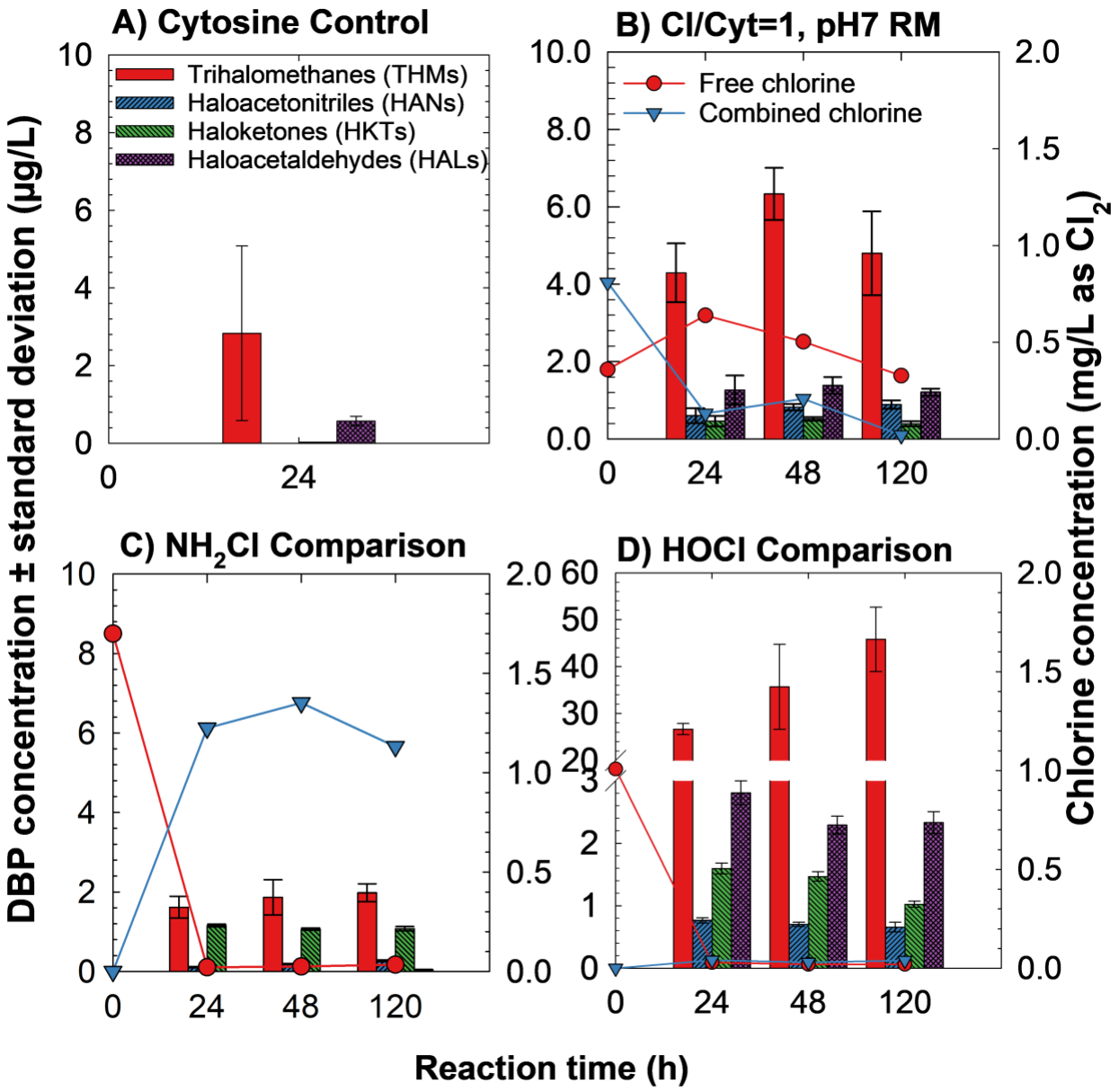

Figure S3. DBP family concentrations (left-hand y-axis) observed to be produced by the (A) cytosine control(50 µM cytosine, 2 mg/L as C SRHA, 5 mM phosphate buffer); (B) *N*-chlorocytosine formed under the reaction conditions: Cl/Cyt=1, pH7 (50 µM cytosine, 50 µM NaOCl, 5 mM phosphate buffer,2 mg/L as C SRHA added after 2 h); (C) monochloramine comparable to the *N*-chlorocytosine concentration reacted with SRHA (19 µM NH_2Cl,2 mg/L as C SRHA); (D) HOCl comparable to the *N*-chlorocytosine concentration reacted with SRHA (19 µM

NaOCl, 2 mg/L as C SRHA, 5 mM phosphate buffer). Error bars represent standard deviation between individual DBPs in the family. The concentrations of free and combined chlorine over the 5 d are also represented (right-hand y-axis) measured by the DPD method.

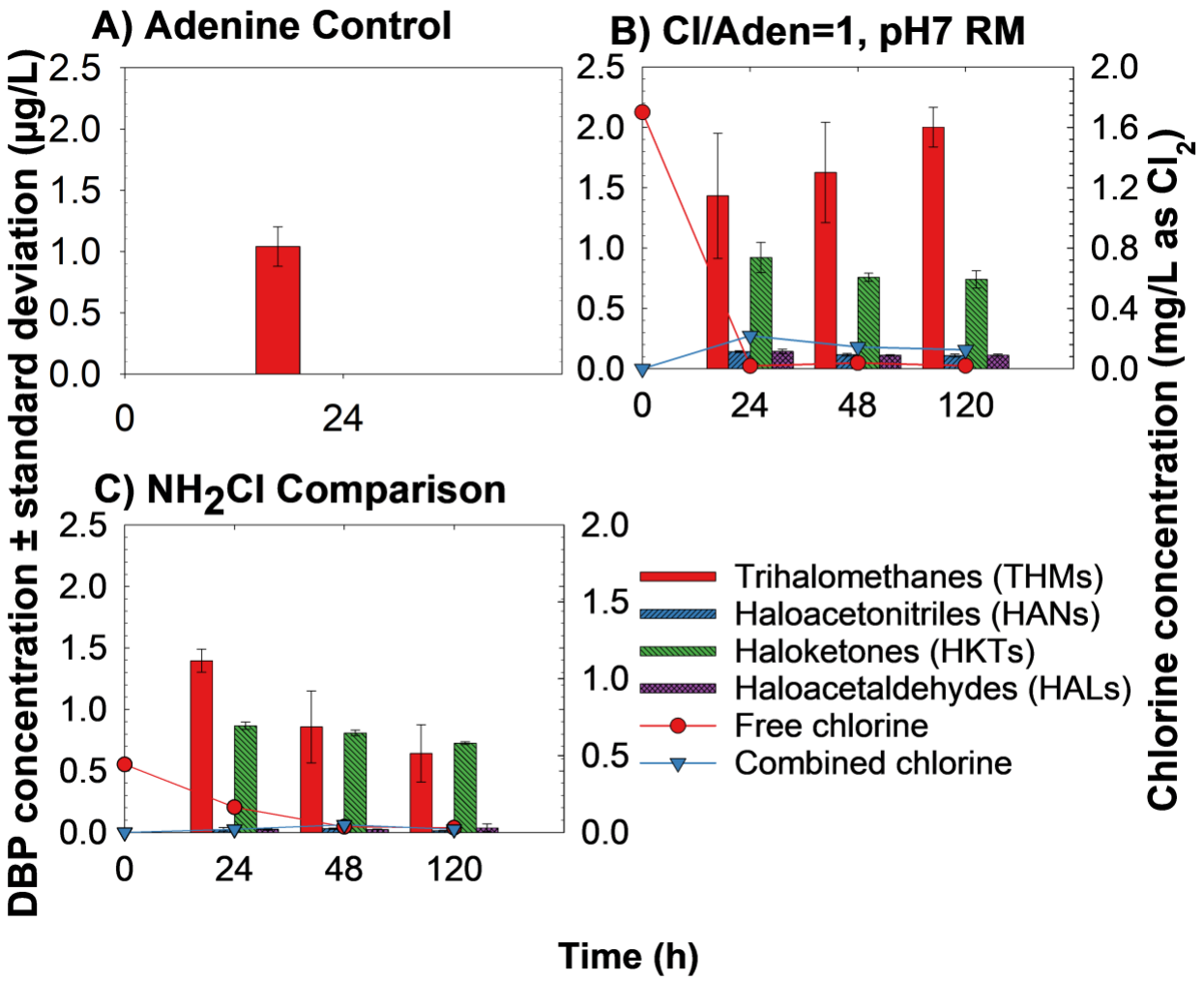

Figure S4. DBP family concentrations (left-hand y-axis) observed to be produced by the (A) adenine control (50 µM adenine, 2 mg/L as C SRHA, 5 mM phosphate buffer); (B) *N*-chloroadenine formed under the reaction conditions: Cl/Aden1, pH7 (50 µM adenine, 50 µM NaOCl, 5 mM phosphate buffer); (C) monochloramine comparable to the *N*-chloroadenine concentration reacted with SRHA (6.7 µM NH_2Cl, 2 mg/L as C SRHA, 5 mM phosphate buffer). Error bars represent standard deviations between individual DBPs in the family. The concentrations of free and combined chlorine over the 5 d are also represented (right-hand y-axis) measured by the DPD method.

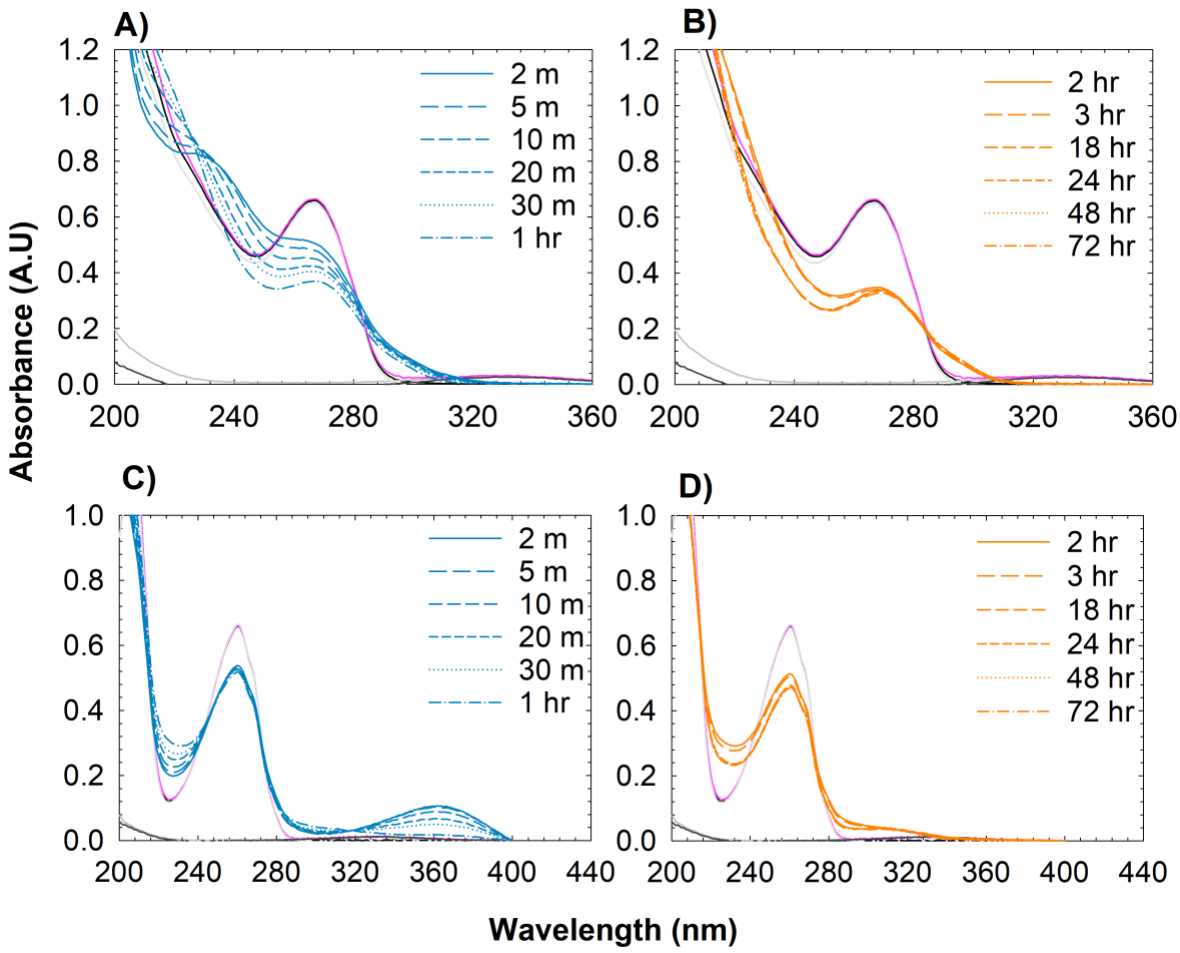

Figure S5. Reaction monitoring by UV-Visible spectrophotometry split by time points to improve visualization. The reaction progress is shown for: Br/Cyt = 1, pH 7 t = 2 min–1 hour (A), Br/Cyt = 1, pH 7 t = 2–72 hours (B), Br/Aden = 1, pH 7 t = 2 min–1 hour (C), and Br/Aden = 1, pH 7 t = 2–72 hours (D).

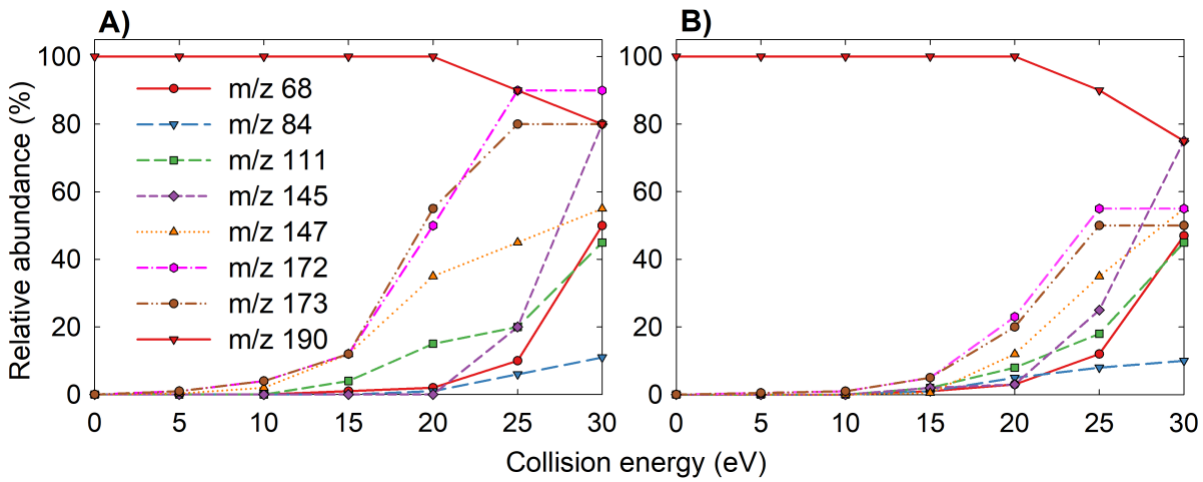

Figure S6. Relative abundances of product ions formed during collision-induced dissociation (CID) in ES+ mode of the *m/z* 190 pre-cursor ion corresponding to protonated 5-bromocytosine. (A) 5-bromocytosine standard and (B) the Br/Cyt=1, pH7 reaction mixture extract, shown as a function of collision energy (0-30 eV, 5 eV increments). Abundances were normalized to the base peak observed at each collision energy (*m/z* 190 for all). Data acquired over *m/z* 50 – 193 using a CID gas pressure of 1.5 mTorr.

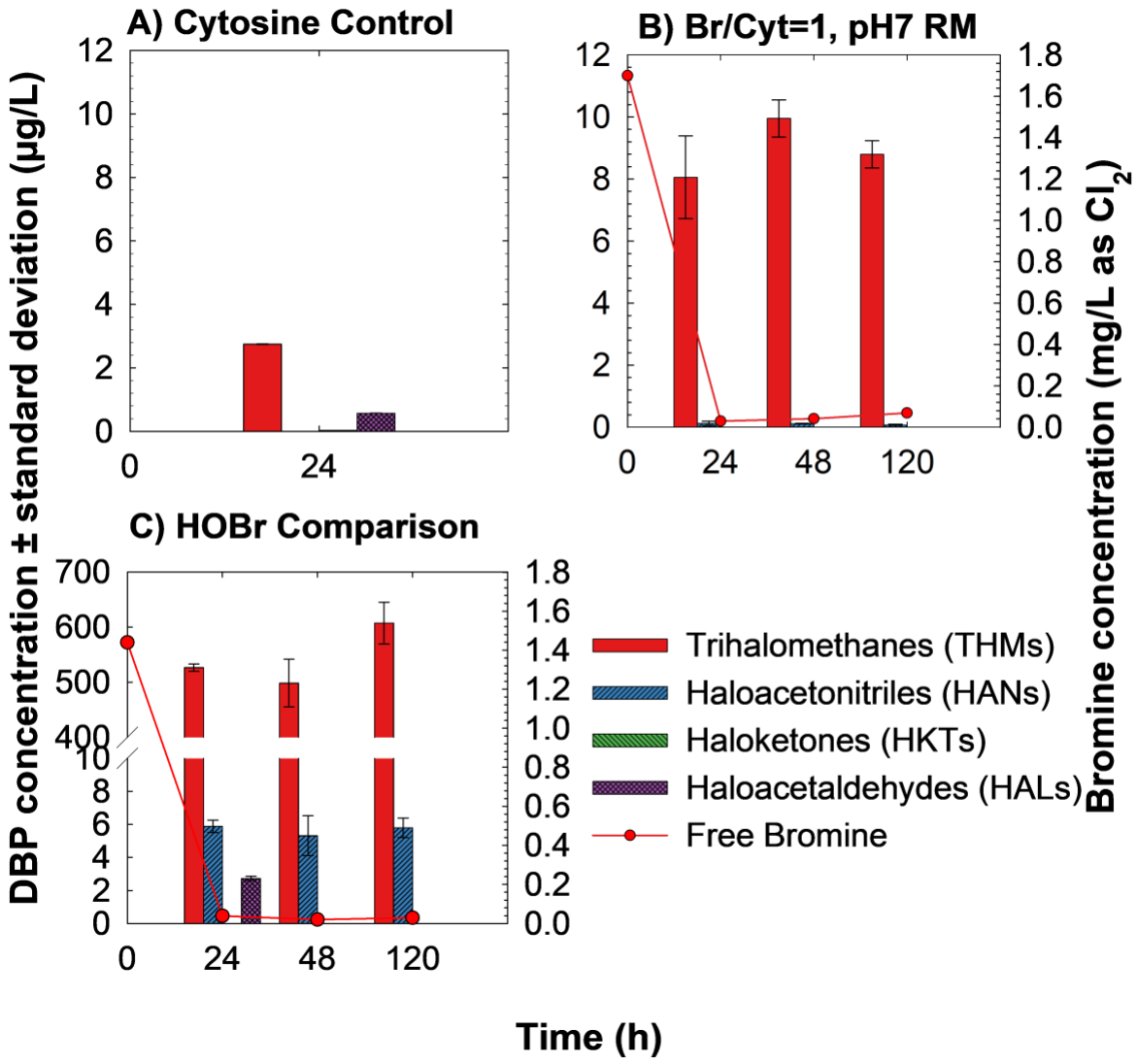

Figure S7. DBP family concentrations (left-hand y-axis) observed to be produced by the (A) cytosine control (50 µM cytosine, 2 mg/L as C SRHA, 5 mM phosphate buffer); (B) the bromocytosine formed under the reaction conditions: Br/Cyt=1, pH7 (50 µM cytosine, 50 µM HOBr, 5 mM phosphate buffer); and (C) a HOBr comparison reaction (50 µM HOBr + 2 mg/L as C SRHA). Error bars represent standard deviations between individual DBPs in the family. The concentration of free bromine over the 5 d is also represented (right-hand y-axis) measured by the DPD method.

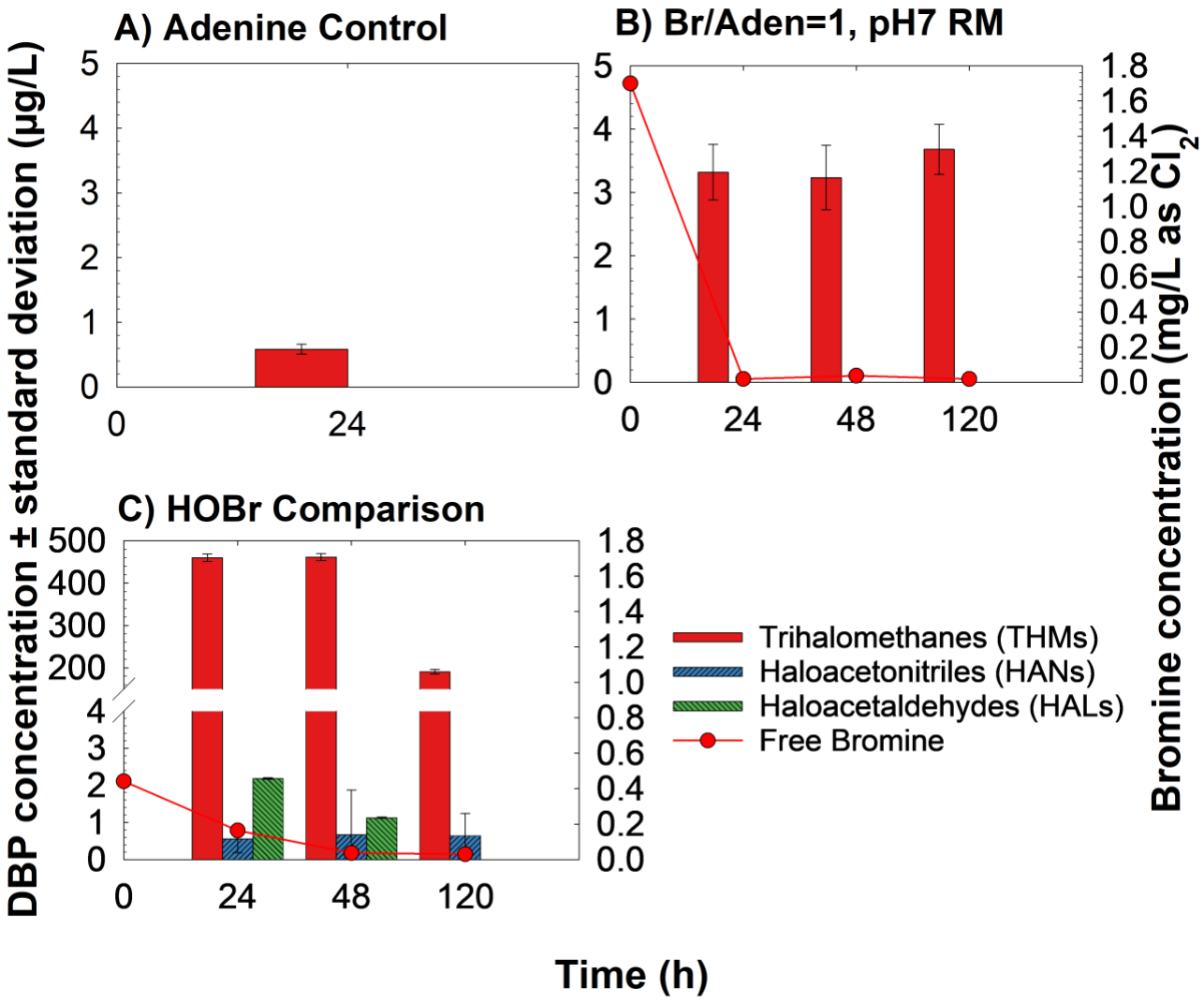

Figure S8. DBP family concentrations (left-hand y-axis) observed to be produced by the (A) adenine control (50 µM adenine, 2 mg/L as SRHA, 5 mM phosphate buffer); (B) the bromoadenine formed under the reaction conditions: Br/Aden1, pH7 (50 µM adenine, 50 µM HOBr, 2 mg/L as C SRHA, 5 mM phosphate buffer); and (C) a HOBr comparison reaction (50 µM HOBr, 2 mg/L as C SRHA, 5 mM phosphate buffer). Error bars represent standard deviations between individual DBPs in the family. The concentration of free bromine over the 5 d is also represented (right-hand y-axis) measured by the DPD method.