**Appendix A. Supplementary Information**

# Three-step effluent chlorination increases disinfection efficiency and reduces DBP formation and toxicity

Yu Li [a], Xiangru Zhang [a,*], Mengting Yang [b,*], Jiaqi Liu [a], Wanxin Li [a], Nigel J.D. Graham [c], Xiaoyan Li [d], Bo Yang [b]

[a]*Department of Civil and Environmental Engineering, The Hong Kong University of Science and Technology, Hong Kong, China*

[b]*College of Chemistry and Environmental Engineering, Shenzhen University, Shenzhen 518060, China*

[c]*Department of Civil and Environmental Engineering, Imperial College London, London, UK*

[d]*Department of Civil Engineering, The University of Hong Kong, Hong Kong, China*

*Corresponding authors.

E-mail addresses: xiangru@ust.hk (X. Zhang), yangmt@szu.edu.cn (M. Yang).

The supplementary information consists of 14 pages, 3 tables and 6 figures.

**Contents**



**Table S1** Characteristics of the primary saline sewage effluent samples collected on September 1 and December 1, 2015.

**Table S2** Residual chlorine concentrations in one-step and three-step chlorination after a given contact time. (a) Each primary sewage effluent sample was chlorinated with a total chlorine dose of 4.0 mg/L as $Cl_2$ and a total contact time of 30 min. (b) Each primary sewage effluent sample was chlorinated with a total chlorine dose of 6.0 mg/L as $Cl_2$ and a total contact time of 15 min.

**Table S3** Developmental toxicity of the primary sewage effluent samples (collected on different days) with one-step and three-step chlorination with a total chlorine dose of 6.0 mg/L as $Cl_2$ and a total contact time of 15 min.

**Fig. S1.** Disinfection efficiencies of the primary sewage effluent sample chlorinated by dosing 1.0−6.0 mg/L NaOCl as $Cl_2$ for a 15- or 30-min contact time. The dashed red line represents the sewage effluent discharge standard. Each error bar indicates the standard deviation of triplicate measurements.

**Fig. S2.** Effect of $KNO_3$ rinsing volume on the TOX measurement. The red dashed line represents the control sample and the black curve represents the simulated saline water sample with various $KNO_3$ rinsing volumes. Each datum presents the mean of duplicate measurements and the difference between the mean and the measured value.

**Fig. S3.** (a) *Escherichia coli* concentrations in the primary sewage effluent samples without or with

one-step and three-step chlorination. (b) Disinfection efficiencies of the primary sewage effluent samples with one-step and three-step chlorination. The primary sewage effluent samples, collected on different days, were chlorinated with a total chlorine dose of 6.0 mg/L as $Cl_2$ and a total contact time of 15 min. Each error bar indicates the standard deviation of triplicate measurements.

**Fig. S4.** Decay of the residual free chlorine concentration in the primary sewage effluent by dosing 4.0 mg/L NaOCl as $Cl_2$ (one-step chlorination). Each error bar indicates the standard deviation of triplicate measurements.

**Fig. S5.** TOCl and TOBr concentrations in the primary sewage effluent samples (collected on different days) with one-step and three-step chlorination with a total chlorine dose of 6.0 mg/L as $Cl_2$ and a total contact time of 15 min. Each error bar indicates the difference between the mean and the measured value.

**Fig. S6.** Developmental toxicity of the primary sewage effluent samples with one-step chlorination (○) and three-step chlorination (◇) with a total chlorine dose of 6.0 mg/L as $Cl_2$ and a total contact time of 15 min. Different charts represent samples collected on different days. Each datum presents the mean of duplicate measurements and the difference between the mean and the measured value.

**1. Characterization of primary saline sewage effluent samples**

The undisinfected sewage effluent samples collected on September 1 and December 1, 2015 were characterized for better understanding the correlation among disinfection efficiency, DBP formation, and toxicity. The results were summarized in Table S1. Ammonia, pH, bromide concentration, salinity and dissolved organic carbon were measured with a flow injection analysis system (8500 Series, Lachat), a pH meter, an ion chromatograph (Shimadzu, Japan), a portable refractometer and TOC analyzer (Shimadzu, Japan), respectively. The ultraviolet (UV) absorbance was measured at 254 nm with a 1-cm quartz cuvette by using a spectrophotometer (Lambda 25, Perkin Elmer, USA). The concentration of total suspended solids was obtained from the Hong Kong Drainage Service Department (HKDSD, 2016).

**2. Total organic halogen (TOX) measurement**

The measurement of total organic chlorine (TOCl) and total organic bromide (TOBr) followed the procedure in previous studies (Hua and Reckhow, 2007; Li et al., 2010; Kristiana et al., 2015). As high levels of salinity (approximately 2.0%) were observed in the primary saline sewage effluent samples, the inorganic halides adsorbed on the activated carbon may not be efficiently removed with a 10-mL $KNO_3$ rinsing solution (5000 mg/L $KNO_3$ as $NO_3^-$), which may seriously interfere with the determination of TOX. To accurately measure the TOX concentration, the $KNO_3$ rinsing volume should be optimized. Aliquots of a simulated saline water sample were prepared by adding 20 g/L NaCl and 100 μg/L 2,4,6-trichlorophenol (TCP) as Cl to ultrapure water. The selected NaCl concentration was based on the salinity level in the primary saline sewage effluent (Table S1) and the selected TCP concentration was based on TOCl (representing the formation of chlorinated DBPs) in the chlorinated primary effluent sample. Then, 80-mL aliquots of each simulated saline water sample were acidified to pH 2 with nitric acid and then passed through two consecutive activated carbon columns (TXA03C, Mitsubishi Chemical Analytech). After adsorption, the activated carbon columns were rinsed with different volumes (10, 20, 30, 40, 50, 60, 70, and 80 mL) of the $KNO_3$ solution (5000 mg/L $KNO_3$ as $NO_3^-$) and were subsequently subjected to pyrolysis at 1000 ºC with an AQF-100 automatic quick furnace (Mitsubishi Chemical Analytech). The hydrogen halide and halogen gases produced from pyrolysis were absorbed in ~7 mL of ultrapure water. Then, the absorption solution was diluted to 8 mL with ultrapure water. An off-line ICS-3000 ion chromatography system with

conductivity detection (Dionex, Sunnyvale, CA) equipped with an IonPac AS19 analytical column (4 × 250 mm) and an AG guard column (4 × 50 mm) was used to analyze the chloride ion in the absorption solution. A control sample, without the addition of NaCl, was prepared according to the aforementioned preparation steps with a 10-mL rinsing volume of the $KNO_3$ solution. Duplicate measurements were conducted for each sample.

Fig. S2 shows the effect of the $KNO_3$ rinsing volume on the measured TOX concentration of a simulated saline water sample. The red dashed line represents the TOCl concentration in the control sample, which was 77.6 (±2.3) μg/L as Cl. The black curve shows the TOCl concentration with different $KNO_3$ rinsing volumes. The TOCl concentration decreased markedly with increasing the $KNO_3$ rinsing volume from 10 to 40 mL. The relatively high measured concentrations of TOCl in the simulated saline sample with 10, 20, and 30 mL of the $KNO_3$ rinsing solution were contributed to the ineffective removal of chloride by the $KNO_3$ solution. With further increase of the $KNO_3$ rinsing volume from 40 to 80 mL, the measured TOCl concentrations in the simulated saline water samples became stable and were approximately equal to the TOCl concentration in the control sample. Therefore, 40 mL of the $KNO_3$ rinsing solution was selected for the TOX measurement in this study.

To determine the concentrations of TOCl and TOBr in the primary saline sewage effluent samples with one-step and three-step chlorination, effluent samples were prepared according to the aforementioned preparation steps. Briefly, 80-mL aliquots of each dechlorinated sample were acidified to pH 2 with nitric acid and passed through two consecutive activated carbon columns. After adsorption, the activated carbon columns were rinsed with 40 mL of the $KNO_3$ solution and were subsequently subjected to pyrolysis at 1000 ºC. The hydrogen halide and halogen gases were absorbed in 8 mL of ultrapure water. An ion chromatography system with conductivity detection was used to analyze the chloride and bromide ions in the absorption solution. Duplicate measurements were conducted for each sample.

**3. Polychaete culturing and development toxicity**

Stock cultures of *Platynereis dumerilii* were maintained according to previous studies (Dorresteijn, 1990; Hutchinson et al., 1995), and the bioassay was conducted per Yang and Zhang's (2013) procedure. Briefly, the worms were cultivated in the seawater at 19 ºC, and fed on a composed diet of algae, chopped spinach, and fish flakes. Breeding was controlled by

photoperiod manipulation providing 16 h (~300 lux) of light and 8 h of darkness. Sexually mature male and female were allowed to spawn naturally in 20 mL of the seawater. As fertilization was confirmed, the developing embryos were transferred to an incubation glass basin containing 200 mL of the seawater. The developmental toxicity tests were conducted with the embryos at 12 h postfertilization. The developing embryos were transferred into a 1.6-cm tissue culture test plate that contained the required concentrated sample stock solution or seawater (as control sample). Then each test solution was diluted to 2 mL by adding seawater. The embryos (~75 embryos/mL) were allowed to develop for a further 12 h. By 24 h post-fertilization, normal embryos were expected to reach the first larval stage with the characteristics of four fat droplets, swimming activity, and surface cilia. Abnormal embryos lack one or serval of these characteristics. The numbers of normal and total embryos in each test solution were checked with the aid of an inverted microscope (magnification by 40 times), and the normal developmental percentage was calculated. By plotting the curve of the percent normal development versus the concentration factor (CF) of the sewage effluent sample (i.e., a CF–response curve), the $EC_{50}$ value (the CF at which the normal development percentage was 50% of that in the seawater control) was obtained via regression analysis using the software SigmaPlot 12. Duplicate measurements were conducted for each sample.

**4. Determination of residual chlorine**

Owing to the high concentration of ammonia (22.6 mg/L $NH_4^+$ as N) in the primary sewage effluent, chlorine reacted rapidly with ammonia to form monochloramine. In the theory of chemical kinetics, the reaction of chlorine and ammonia to form monochloramine is very fast (Morris, 1967). In practice, however, the overall reaction rate of this reaction in a reactor is controlled by mixing which transports the reactants to each other, and thus chlorine might exist for a short time before being converted to monochloramine. Wolfe et al. (1984) indicated that for sub-optimal treatment conditions, such as insufficient mixing, chlorine may co-exist with monochloramine for a short time (usually for a few minutes) prior to complete monochloramine formation. Accordingly, the chlorine concentration obtained from a thermodynamics or kinetics calculation, or measured by a stopped-flow system, might not be representative for the reaction conditions in the disinfection efficiency test. The concentration of residual chlorine needed to be accurately monitored with time. Because a high concentration of monochloramine (> 0.5 mg/L as

$Cl_2$) may break into chlorine and seriously interfere with the detection of chlorine, a modified procedure was used in this study for accurately measuring chlorine, involving the addition of a thioacetamide solution (APHA et al., 2012). Adding thioacetamide can terminate completely further reaction with monochloramine in the chlorine test (APHA et al., 2012).

To initiate the residual chlorine measurement, a series of 100-mL aliquots of an unchlorinated primary sewage effluent sample, a phosphate buffer–DPD (v/v, 1:1) mixed solution, and a 0.25% thioacetamide solution (w/w) were freshly prepared prior to the test. Then, a 100-mL aliquot of the sewage effluent sample was chlorinated by dosing 4.0 mg/L as $Cl_2$. After a given contact time (controlled by a timer), 10 mL of the buffer–DPD mixed solution and 0.5 mL of the 0.25% thioacetamide solution were successively added to the aliquot. The absorbance of the produced pink color was read immediately by a spectrophotometer at 515 nm and the chlorine concentration was quantified by a calibration curve.

Fig. S4 presents the decay of the residual chlorine concentration of a primary effluent sample by dosing a chlorine dose of 4.0 mg/L as $Cl_2$. The concentration of chlorine decreased quickly and chlorine disappeared within 10 s after it was dosed. Thus, chlorine can exist for a short time prior to complete monochloramine formation.

**5. One-tailed *t*-statistical significance test**

For each primary effluent sample collected on a day, there are two independent groups of $EC_{50}$ values (i.e., one group for one-step chlorination, and the other group for three-step chlorination), and each group of $EC_{50}$ values follows a normal distribution. To test whether the average $EC_{50}$ value of one group of data is significantly higher than that of the other, the statistic value ($t_s$) can be calculated with the following equation (Tao, 1994; Li et al., 2011):

$$t_s = \frac{\left|X_1 - X_2\right|}{\sqrt{\frac{(n_1-1)S_1^{\,2} + (n_2-1)S_2^{\,2}}{n_1+n_2-2} \times \frac{n_1+n_2}{n_1 n_2}}}$$

where $n_1$ and $n_2$ are the numbers of data in groups 1 (i.e., the primary effluent sample with one-step chlorination) and group 2 (i.e., the primary effluent sample with three-step chlorination), respectively; $X_1$ and $X_2$ are the average $EC_{50}$ values of data in groups 1 and 2, respectively;

$S_1$ and $S_2$ are the variances of data in groups 1 and 2, respectively.

For one-tailed *t*-statistical significance test with a statistical significance level ($\alpha$) of 0.05 or 0.10, the value of $t_{2\alpha[n1+n2-2]}$ can be found in student's *t*-table (Tao, 1994).

When $t_s > t_{2\alpha[n1+n2-2]}$, the average value of one group of data is significantly higher than that of the other; the probability (*p*) that the difference happened by chance is small, and *p* is less than the critical $\alpha$ level ($p < \alpha$). When $t_s < t_{2\alpha[n1+n2-2]}$, the average value of one group of data is not significantly higher than that of the other; the probability (*p*) that the difference happened by chance is high, and *p* is greater than the critical $\alpha$ level ($p > \alpha$).

**6. References**


APHA, AWWA, WEF, 2012. Standard Methods for the Examination of Water and Wastewater, twenty-second ed. American Public Health Association, Washington, DC.

Dorresteijn, A.W.C., 1990. Quantitative analysis of cellular differentiation during early embryogenesis of *Platynereis dummerilii*. Dev. Genes Evol. 199 (1), 14–30.

HKDSD, 2016. Effluent Quality Report of Major Sewage Treatment Works. Hong Kong Drainage Service Department.

Hua, G., Reckhow, D.A., 2007. Comparison of disinfection byproduct formation from chlorine and alternative disinfectants. Water Res. 41 (8), 1667–1678.

Hutchinson, T.H., Jha, A.N., Dixon, D.R., 1995. The polychaete *Platynereis dumerilii* (Audouin and Milne-Edwards): a new species for assessing the hazardous potential of chemicals in the marine environment. Ecotoxicol. Environ. Saf. 31 (3), 271–281.

Kristiana, I., McDonald, S., Tan, J., Joll, C., Heitz, A., 2015. Analysis of halogen-specific TOX revisited: Method improvement and application. Talanta 139, 104–110.

Li, Y., Zhang, X., Shang, C., 2010. Effect of reductive property of activated carbon on total organic halogen analysis. Environ. Sci. Technol. 44, 2105–2111.

Li, Y., Zhang, X., Shang, C., Krasner, S.W., 2011. Evaluation and improvement of total organic bromine analysis with respect to reductive property of activated carbon. Water Res. 45 (3), 1229–1237.

Morris, J. C., 1967. Kinetics of reactions between aqueous chlorine and nitrogen compounds. In: Faust, S. D., Hunter, J. V. (Eds.), Principles and Applications of Water Chemistry. John Wiley & Sons, New York, NY, pp. 23–53.

Tao, S., 1994. Applied Mathematical Statistics. China Environmental Science press, Beijing.

Wolfe, R.L., Ward, N.R., Olson, B.H., 1984. Inorganic chloramines as drinking water disinfectants: a review. J. Am. Water Works Assoc. 76 (5), 74–88.

Yang, M., Zhang, X., 2013. Comparative developmental toxicity of new aromatic halogenated DBPs in a chlorinated saline sewage effluent to the marine polychaete *Platynereis dumerilii*. Environ. Sci. Technol. 47 (19), 10868–10876.

**Table S1**

Characteristics of the primary saline sewage effluent samples collected on September 1 and December 1, 2015.

| | Sampling date | |
|---|---|---|
| | Sep 1, 2015 | Dec 1, 2015 |
| pH | 6.85 | 7.10 |
| $NH_4^+$ (mg/L as N) | 22.4 | 22.8 |
| $Br^-$ (mg/L) | 28.9 | 29.3 |
| Salinity (%) | 1.9 | 2.0 |
| Dissolved organic carbon (mg/L as C) | 23.0 | 29.7 |
| $UV_{254}$ absorbance ($cm^{-1}$) | 0.189 | 0.211 |
| Total suspended solids (mg/L) | 43.0 | 45.0 |

**Table S2**

Residual chlorine concentrations in one-step and three-step chlorination after a given contact time. (a) Each primary sewage effluent sample was chlorinated with a total chlorine dose of 4.0 mg/L as $Cl_2$ and a total contact time of 30 min. (b) Each primary sewage effluent sample was chlorinated with a total chlorine dose of 6.0 mg/L as $Cl_2$ and a total contact time of 15 min.

| (a) Effluent sample chlorinated with 4.0 mg/L for 30 min | | Residual chlorine ± SD [a] (mg/L as $Cl_2$) | Chlorine consumption ± SD [a] (mg/L as $Cl_2$) |
|---|---|---|---|
| Effluent collected on Sep 1, 2015 | 1-step | 2.43±0.09 | 1.57±0.09 |
| | 3-step | 2.62±0.04 | 1.38±0.04 |
| Effluent collected on Dec 1, 2015 | 1-step | 2.60±0.03 | 1.40±0.03 |
| | 3-step | 2.65±0.04 | 1.35±0.04 |

| (b) Effluent sample chlorinated with 6.0 mg/L for 15 min | | Residual chlorine ± SD [a] (mg/L as $Cl_2$) | Chlorine consumption ± SD [a] (mg/L as $Cl_2$) |
|---|---|---|---|
| Effluent collected on Sep 1, 2015 | 1-step | 3.23±0.15 | 2.77±0.15 |
| | 3-step | 3.34±0.10 | 2.66±0.10 |
| Effluent collected on Dec 1, 2015 | 1-step | 3.73±0.03 | 2.27±0.03 |
| | 3-step | 3.98±0.07 | 2.02±0.07 |

[a] Standard deviation of triplicate measurements.

**Table S3**

Developmental toxicity of the primary sewage effluent samples (collected on different days) with one-step and three-step chlorination with a total chlorine dose of 6.0 mg/L as $Cl_2$ and a total contact time of 15 min.

| Effluent sample | | $EC_{50}$±SD [a] | $R^2$ [b] |
|---|---|---|---|
| Effluent collected on Feb 27, 2014 | 1-step | 3.33±0.05 | 0.992 |
| | 3-step | 3.72±0.06 | 0.993 |
| Effluent collected on Apr 17, 2015 | 1-step | 3.13±0.01 | 0.990 |
| | 3-step | 3.58±0.01 | 0.993 |
| Effluent collected on Sep 1, 2015 | 1-step | 3.76±0.23 | 0.993 |
| | 3-step | 3.98±0.08 | 0.984 |
| Effluent collected on Dec 1, 2015 | 1-step | 2.68±0.17 | 0.987 |
| | 3-step | 3.03±0.10 | 0.991 |

[a] the standard deviation of duplicate measurements for each chlorinated primary saline effluent sample; [b] the regression coefficient of the concentration factor–response curve for each chlorinated primary saline effluent sample.

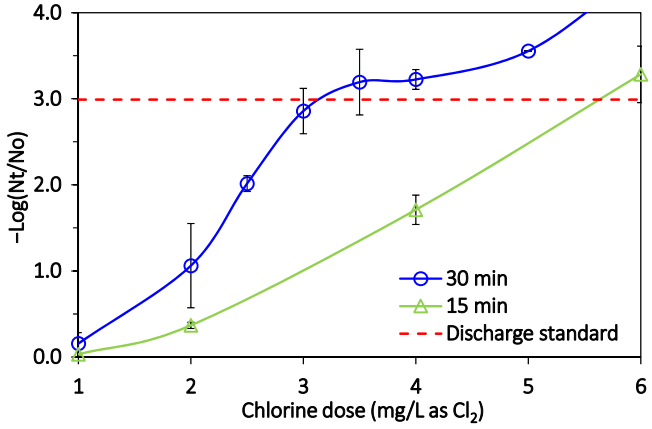


**Fig. S1.** Disinfection efficiencies of the primary sewage effluent sample chlorinated by dosing 1.0−6.0 mg/L NaOCl as $Cl_2$ for a 15- or 30-min contact time. The dashed red line represents the sewage effluent discharge standard. Each error bar indicates the standard deviation of triplicate measurements.

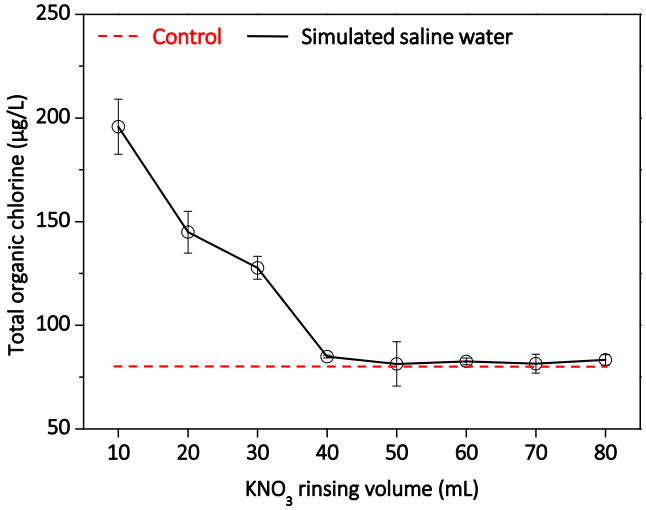


**Fig. S2.** Effect of $KNO_3$ rinsing volume on the TOX measurement. The red dashed line represents the control sample and the black curve represents the simulated saline water sample with various $KNO_3$ rinsing volumes. Each datum presents the mean of duplicate measurements and the difference between the mean and the measured value.

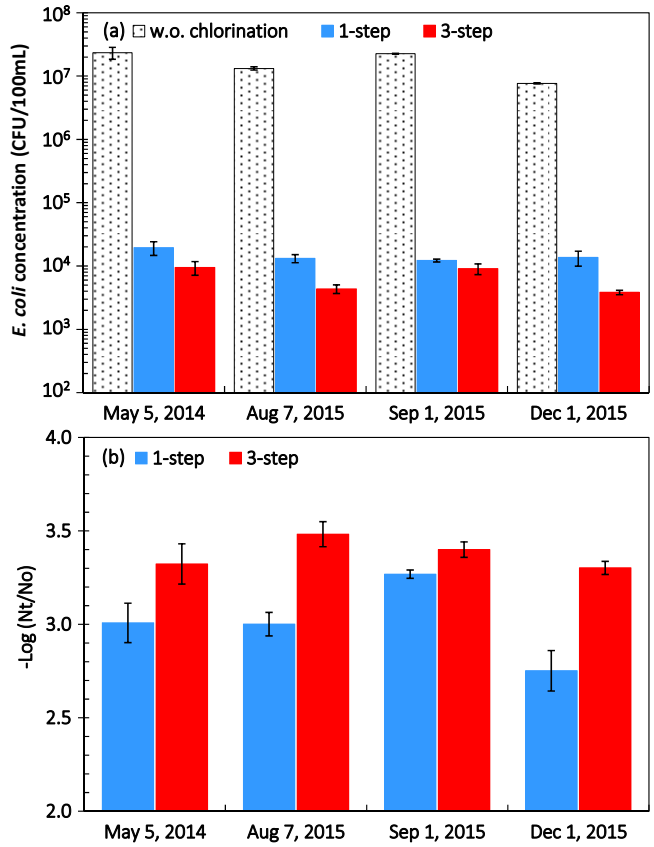


**Fig. S3.** (a) *Escherichia coli* concentrations in the primary sewage effluent samples without or with one-step and three-step chlorination. (b) Disinfection efficiencies of the primary sewage effluent samples with one-step and three-step chlorination. The primary sewage effluent samples, collected on different days, were chlorinated with a total chlorine dose of 6.0 mg/L as $Cl_2$ and a total contact time of 15 min. Each error bar indicates the standard deviation of triplicate measurements.

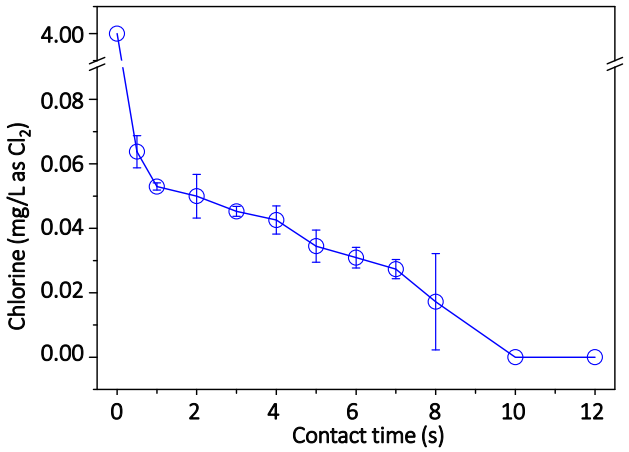


**Fig. S4.** Decay of the residual free chlorine concentration in the primary sewage effluent by dosing 4.0 mg/L NaOCl as $Cl_2$ (one-step chlorination). Each error bar indicates the standard deviation of triplicate measurements.

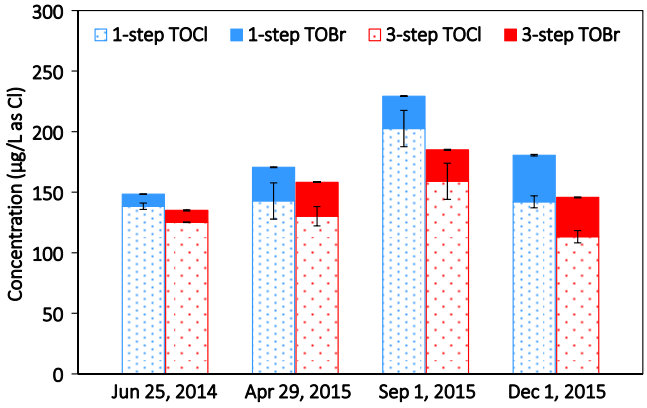


**Fig. S5.** TOCl and TOBr concentrations in the primary sewage effluent samples (collected on different days) with one-step and three-step chlorination with a total chlorine dose of 6.0 mg/L as $Cl_2$ and a total contact time of 15 min. Each error bar indicates the difference between the mean and the measured value.

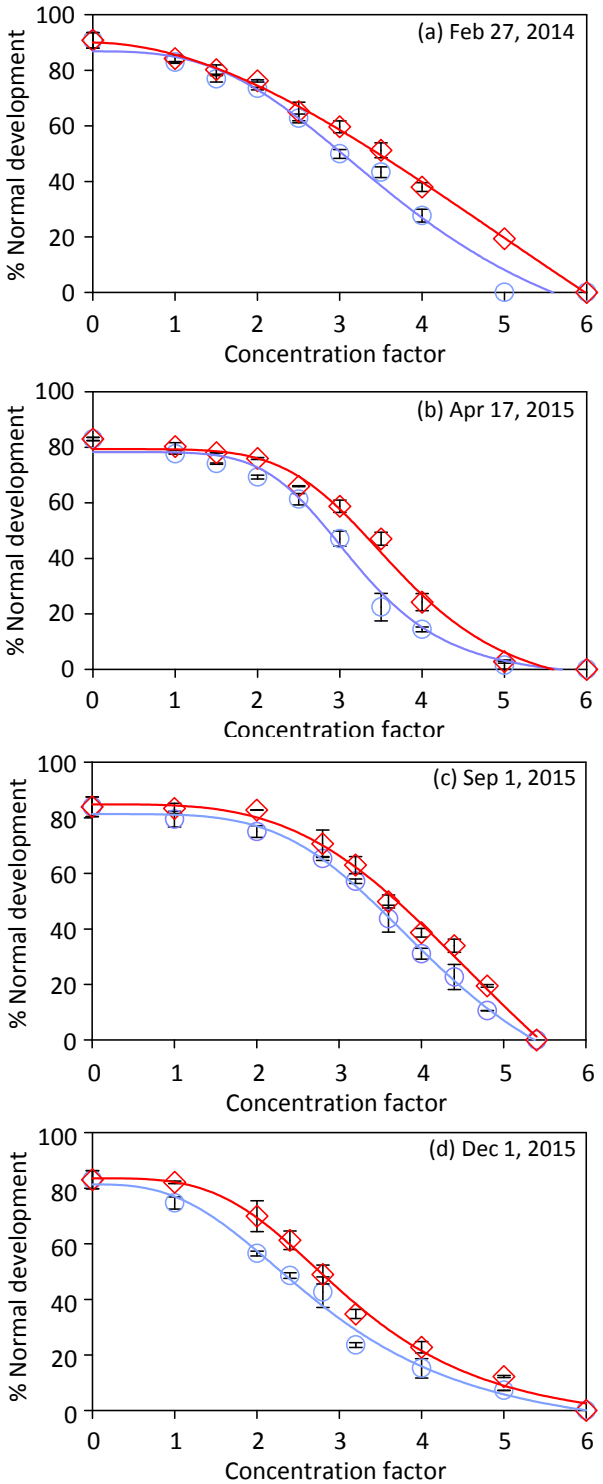


**Fig. S6.** Developmental toxicity of the primary sewage effluent samples with one-step chlorination (◯) and three-step chlorination (◇) with a total chlorine dose of 6.0 mg/L as $Cl_2$ and a total contact time of 15 min. Different charts represent samples collected on different days. Each datum presents the mean of duplicate measurements and the difference between the mean and the measured value.