



Specific and total *N*-nitrosamines formation potentials of nitrogenous micropollutants during chloramination

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ABSTRACT

N-nitrosamines are a group of potent human carcinogens that can be formed during oxidative treatment of drinking water and wastewater. Many tertiary and quaternary amines present in consumer products (e.g., pharmaceuticals, personal care and household products) are known to be *N*-nitrosodimethylamine (NDMA) precursors during chloramination, but the formation of other *N*-nitrosamines has been rarely studied. This study investigates the specific and total *N*-nitrosamine (TONO) formation potential (FP) of various precursors from nitrogen-containing micropollutants (chlorhexidine, metformin, benzalkonium chloride and cetyltrimethylammonium chloride) and tertiary and quaternary model amines (trimethyl amine, *N,N*-dimethylbutyl amine, *N,N*-dimethylbenzyl amine and tetramethyl ammonium). All the studied nitrogenous micropollutants displayed quantifiable TONO FP, with molar yields in the range 0.04–11.92%. However, the observed TONO pools constituted mostly of uncharacterized species, not included in US-EPA 8270 *N*-nitrosamines standard mix. Only the quaternary ammonium compound benzalkonium chloride showed quantifiable NDMA FP (0.56% molar yield), however, explaining only a minor fraction of the observed TONO FP. The studied model amines showed molar NDMA yields from 0.10% (trimethyl amine) to 5.05% (*N,N*-dimethylbenzyl amine), very similar to the molar TONO yields. The comparison of the FPs of micropollutants and model compounds showed that the presence of electron donating functional groups (such as a benzyl group) in tertiary and quaternary amine precursors leads to a higher formation of NDMA and uncharacterized *N*-nitrosamines, respectively. LC-qTOF screening of a list of proposed *N*-nitrosamine structures has enabled to identify a novel *N*-nitrosamine (*N*-nitroso-*N*-methyl dodecylamine) from the chloramination of benzalkonium chloride. This finding supports the hypothesis that different functional groups in quaternary amines can act as leaving groups during chloramination and form differing *N*-nitrosamine structures at significant yield. Molar TONO yields determined for micropollutants were finally validated under experimental conditions closer to real water matrices, confirming their representativeness also for lower concentration ranges.

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1. Introduction

N-nitrosamines are a group of emerging disinfection by-products that can be formed during chloramination, chlorination and ozonation of drinking water and wastewater (Krasner et al., 2013; Mitch and Sedlak, 2004; Richardson and Ternes, 2014).

Several *N*-nitrosamines are classified as probable human carcinogens and mutagens, with low nanogram-per-litre drinking water concentrations associated with a 10^{-6} lifetime excess cancer risk (IRIS US EPA, 2018; Mitch et al., 2003). US-EPA listed five *N*-nitrosamines on the Contaminant Candidate List 4 (CCL4) as contaminants potentially occurring in public water systems (US EPA CCL4, 2014).

Previous studies (Kemper et al., 2010; Le Roux et al., 2011; Mitch and Sedlak, 2004; Zeng and Mitch, 2015) showed that various consumer products, such as pharmaceuticals, personal care and household products, play an important role in the formation of *N*-

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nitrosamines. These classes of compounds are typically present in municipal wastewater effluents, wherefrom they are discharged into the aquatic environment and may reach water resources used for drinking water production (Schwarzenbach et al., 2006). Zeng and Mitch (2015) found that domestic greywater and blackwater streams are significant sources of chloramine-reactive and ozone-reactive *N*-nitrosamine precursors. Even though specific precursors have not been characterized, *N*-nitrosamine formation was associated with the presence of shampoos, handsoaps, dishsoaps and pharmaceuticals. Specific *N*-nitrosamine precursors may include tertiary or quaternary amines, being common macro-constituents of many consumer products (Dai and Mitch, 2013; Shen and Andrews, 2011a).

Various tertiary amines commonly present in herbicides and in pharmaceutical and personal care products were found to be *N*-nitrosodimethylamine (NDMA) precursors during chloramination, showing molar conversion yields varying from <1% up to 60–90% for ranitidine (Le Roux et al., 2011; Lee et al., 2007; Mitch and Schreiber, 2008; Mitch and Sedlak, 2004; Sacher et al., 2008; Schmidt and Sacher, 2006; Selbes et al., 2013; Shen and Andrews, 2011a, 2011b; Spahr et al., 2016). Various mechanisms have been proposed for NDMA formation during chloramination of tertiary amines (Choi and Valentine, 2002a, 2002b, 2003; Shah et al., 2012; Shah and Mitch, 2011). NDMA formation was initially attributed to the liberation of the dimethylamine (DMA) moiety via reactions of chlorine or monochloramine followed by reactions between DMA and dichloramine (Krasner et al., 2013; Mitch and Schreiber, 2008; Mitch and Sedlak, 2004). Further studies suggested that tertiary amines can also form *N*-nitrosamines without proceeding through a secondary amine intermediate, but through nucleophilic attack of the amine on chloramine (Selbes et al., 2013). Recent studies showed that the stability and the electron distribution of the moieties bound to the DMA group play a key role in NDMA formation and strongly affect its production yields (Shah and Mitch, 2011). In particular, the presence of an electron-donating group close to the DMA moiety can increase the electron density on the nitrogen atom, enhancing a nucleophilic attack on the chloramines (Le Roux et al., 2011; Selbes et al., 2013; Shen and Andrews, 2011a).

Compared to tertiary amines, only few studies investigated the formation of *N*-nitrosamines from specific quaternary ammonium compounds (QACs). Various quaternary ammonium cationic polymers (e.g., polyDADMAC, polyamine, polyacrylamide) used for water coagulation and flocculation are known to promote NDMA formation during chloramination, due to direct polymer nitrosation and mainly the formation of intermediate polymer degradation products (Cornwell et al., 2015; Park et al., 2009b, 2015; Zeng et al., 2016). Kemper et al. (2010) found that NDMA is formed during chloramination of different QACs used as surfactants and disinfection agents, such as quaternary alkylamines and benzyl trialkylamines, with relatively low molar yields (0.03–0.28%). So far, the mechanism of the formation of *N*-nitrosamines from QACs remains unclear. The full substitution of the nitrogen and the positive charge on the quaternary amine should hinder the reaction with chloramines (Krasner et al., 2013). QACs can contain impurities that might constitute potential *N*-nitrosamines precursors. However, Kemper et al. (2010) have shown that the formation of *N*-nitrosodibutylamine (NDBA) by chloramination of benzyltributylammonium chloride only slightly decreased after purification. This indicates that the QAC itself, rather than impurities, was the dominant *N*-nitrosamine precursor. Even though the reaction pathways have not been defined, the same authors suggested that the NDMA formation from QACs may involve amidogen or chloramino radicals formed from chloramines. Some quaternary amine monomers (e.g., choline, cocoamidopropyl betaine) and polymers (e.g., polyDADMAC, polyquaternium-7, polyacrylamide,

polyamine and DMA-*epi*-DMA) were also found to form NDMA during ozonation (Marti et al., 2015; Padhye et al., 2011), free chlorine and chlorine dioxide disinfection (Park et al., 2009a; Zhang et al., 2014), even though at molar yields lower than those observed for chloramination.

The majority of the studies in the literature deals with the formation of NDMA, being the most often detected *N*-nitrosamine in tap and wastewater (Russell et al., 2012). However, previous studies showed that NDMA is often a minor part of the total *N*-nitrosamines (TONO) pool (Dai and Mitch, 2013; Kulshrestha et al., 2010; Zeng and Mitch, 2015). Analysing 36 drinking water treatment plants and distribution systems, Dai and Mitch (2013) found that NDMA made up only 5% of the TONO pool on a median basis. Zeng and Mitch (2015) observed that the contribution of NDMA to TONO was 1–11% and 3–60% in domestic greywater streams treated by chloramine and ozone, respectively. The same authors suggested that the precursors of the uncharacterized *N*-nitrosamine fraction were associated to common macro-constituents of consumer products, such as QACs present in shampoos and soaps.

While many NDMA precursors were identified in the literature, the determination of the TONO formation potential (FP) of specific model compounds is not a common procedure. The investigations of *N*-nitrosamine formation are typically limited to the species detected by the US-EPA method 521 (US EPA, 2005), while the identity of the other components of the TONO pool remains unclear.

The goal of this study was to investigate the formation of *N*-nitrosamines during chloramination of various nitrogenous compounds that can be discharged to municipal wastewater and may end up in the receiving water bodies. The investigated compounds included two quaternary ammonium compounds used as disinfectants and surfactants (benzalkonium chloride, BZK, a mixture of benzyl trialkylamines, and cetyltrimethylammonium chloride, CTMA, a quaternary alkylamine) and two biguanides (chlorhexidine, CHD, an antiseptic agent used in disinfectants, pharmaceuticals and cosmetics, and metformin, MET, a prescription drug used for treating diabetes).

Laboratory experiments on model solutions were performed to determine the formation potential of total and specific *N*-nitrosamines for the studied precursors as a function of the pH. Besides analyses of TONO and *N*-nitrosamines from the US-EPA 8270 standard mix (US EPA, 2005), high resolution mass spectrometry analyses were carried out to identify the components of the unknown fraction of TONO. The role of the molecular structure of the precursors on *N*-nitrosamine formation was further investigated by model tertiary and quaternary amines (trimethyl amine, *N,N*-dimethylbutyl amine, *N,N*-dimethylbenzyl amine and tetramethyl ammonium) containing functional groups similar to the selected micropollutant moieties.

2. Materials and methods

2.1. Chemicals and reagents

All the experiments were conducted in ultrapure water (Milli-Q, Millipore) buffered with a phosphate buffer (0.5 M) at different pH values (6, 7 or 8). Chlorhexidine dihydrochloride (CHD, >98%), metformin hydrochloride (MET, 99%), benzalkonium chloride (BZK, >95%), cetyltrimethylammonium chloride (CTMA, 25%wt in H₂O), tetramethylammonium chloride (TeMA, >99%), trimethylamine (TMA, 98%), *N,N*-dimethylbenzyl amine (DMBzA, >99%) and *N,N*-dimethylbutyl amine (DMBA, 99%) were purchased from Sigma-Aldrich. The molecular structures and main properties of the selected compounds are provided in Table 1. Concentrated stock solutions of each precursor were prepared in ultrapure water. The

Table 1

Selected micropollutants and model compounds: molecular structure, molecular weight (MW) and experimentally determined pK_a values (references given in the footnotes). For CHD and MET, the pK_a values are referred to the equilibrium $\text{C}=\text{NH}_2^+ \rightleftharpoons \text{C}=\text{NH} + \text{H}^+$.

Compound	Structure	MW (g mol ⁻¹)	pK _a
Micropollutants			
Chlorhexidine dihydrochloride (CHD)		578.4	2.20 ^a 10.30 ^b
Metformin hydrochloride (MET)		165.6	10.27 ^c 2.97 ^d
Benzalkonium chloride (BZK)		283.4–423.4 (average 348.4)	-
Cetyltrimethylammonium chloride (CTMA)		319.4	-
Model compounds			
Tetramethylammonium chloride (TeMA)		109.6	-
Trimethylamine (TMA)		59.1	9.80 ^e
N,N-dimethylbutylamine (DMBA)		101.2	10.19 ^f
N,N-dimethylbenzylamine (DMBzA)		135.2	8.91 ^g

^{a, b} (Agarwal et al., 2012).

^{c, d} (Devi, 2013).

^{e, f, g} ChemIDplus-Toxnet database (<https://chem.nlm.nih.gov/chemidplus/>).

US-EPA 8270 *N*-nitrosamine standard mix, containing *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosomethylethylamine (NMEA), *N*-nitrosomorpholine (NMOR), *N*-nitrosodibutylamine (NDBA), *N*-nitrosopiperidine (NPIP), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosodipropylamine (NDPA) and *N*-nitrosodiphenylamine (NDPhA) at the same mass concentrations (2000 µg mL⁻¹ of each component in dichloromethane), was obtained from Sigma-Aldrich. Monochloramine stock solutions were prepared daily as described by Le Roux et al. (2011) using sodium hypochlorite (NaOCl, 15–20%, Sigma-Aldrich) and ammonium chloride (99.9%, VWR Chemicals). The concentrations of monochloramine (NH₂Cl) and dichloramine (NHCl₂) (see section 2.3) in the stock solutions were measured after preparation; the NHCl₂ concentration was always not quantifiable (see section 2.3). Ascorbic acid, HPLC grade acetonitrile, dichloromethane and methanol were purchased from Sigma-Aldrich.

2.2. Experimental procedures

The self-decay of monochloramine at various initial concentrations (1, 5, 20 and 100 mM) and pH values (6, 7 and 8) was investigated by measuring the NH₂Cl concentration over time (see section 2.3) in buffered ultrapure water. Chloramination experiments were conducted in amber borosilicate bottles at room temperature (23 °C) under two experimental setups (ES), denoted ES1 for high concentrations of precursors and ES2 for more realistic lower concentrations of precursors.

Under ES1, reactions were conducted in 10 mL solutions, composed of variable volumes of precursor stock solutions, sodium phosphate buffer (125 mM in the sample), pre-formed monochloramine stock solution and ultrapure water. The initial concentration of the precursors was set to 0.1 mM for CHD and 0.5 mM for all the other precursors, while the one of the buffer was set to

have a buffer excess with respect to the initial monochloramine concentration. *N*-nitrosamine FP is operationally defined as the maximum *N*-nitrosamine concentration that can be formed from a precursor, using a NH_2Cl concentration and a contact time much higher than applied during drinking water treatment (Krasner et al., 2013). In this study, the experimental conditions to be adopted in the FP tests (i.e., reaction time, initial concentrations of precursors and monochloramine) under ES1 were selected according to a series of preliminary kinetic tests. The kinetics of *N*-nitrosamine formation from CHD, MET, BZK and CTMA at pH 7 were investigated by testing different initial NH_2Cl concentrations (corresponding to 10-, 20-, 40-, 100- and 200-fold molar excess) and reaction times (1, 2, 5, 7 and 10 days) and measuring TONO concentrations and the residual precursor concentrations (only for CHD and BZK) (SI, Text S3). According to the results of the kinetic tests (discussed in section 3.1), FP tests at pH 6, 7 and 8 were conducted with all the precursors (micropollutants and model compounds) at a reaction time of 7 days and a 200-fold molar excess of NH_2Cl relative to the precursor compound; only in the case of BZK, FP tests were performed with a 10-fold NH_2Cl molar excess, assuring the highest *N*-nitrosamine production (according to kinetic test results discussed in section 3.1). The concentrations of TONO and *N*-nitrosamines of the US-EPA 8270 standard mix (see section 2.3) were measured at the end of the FP tests.

In ES2, 7 days FP tests were conducted in 0.5 L solutions, containing deionized water, 1 mM sodium phosphate buffer (pH 8), 100 μM and 0.5 μM initial concentrations of monochloramine and precursor, respectively (corresponding to a 200-fold NH_2Cl molar excess). The TONO concentration was measured after sample pre-concentration by solid phase extraction (see section 2.3).

The molar yield of *N*-nitrosamines formation was calculated as the ratio between the produced molar concentration of the *N*-nitrosamine and the molar concentration of the precursor.

For all the chloramination experiments, reaction solutions were quenched at the end of the pre-determined reaction time by dosing an ascorbic acid stock solution (280 mM) to get a stoichiometric excess relative to the initial NH_2Cl concentration. Quenched solutions were stored at 4 °C in the dark. All the experiments were performed in duplicate.

2.3. Analytical methods

NH_2Cl and NHCl_2 concentrations were determined spectrophotometrically (Shimadzu, UV-1800) using their respective molar extinction coefficients at 245 and 295 nm, respectively and solving the equations expressing absorbance values at the two wavelengths simultaneously (Schreiber and Mitch, 2005). The NHCl_2 concentration is quantifiable only when the ratio between absorbance values at 295 nm and 245 nm is $\geq 3\%$, corresponding to a $\text{NHCl}_2:\text{NH}_2\text{Cl}$ molar ratio $\leq 0.01\%$.

Total *N*-nitrosamine concentrations were measured by a method based on UV photolysis and chemiluminescence measurement of nitric oxide (see Breider and von Gunten (2017) for more details). The chemiluminescence signal was related to the TONO concentration by a standard curve, obtained with different concentrations of the US-EPA 8270 *N*-nitrosamine standard mix (from 0.8 to 16.2 μM of each *N*-nitrosamine). The test solutions in absence of monochloramine were compared with ultrapure water, to verify the absence of a background signal. The calibration was repeated daily before each measurement session. The limit of detection (LOD) and quantification (LOQ) at the 95% confidence level of the analytical method were calculated based on the standard error (SE) and the slope (m) of the calibration curve ($\text{LOD} = 3.3 \times \text{SE}/m$, $\text{LOQ} = 10 \times \text{SE}/m$). Average (\pm standard deviation) LOD and LOQ resulted equal to $0.3 \pm 0.15 \mu\text{M}$ and

$1.0 \pm 0.45 \mu\text{M}$, respectively. For each sample, TONO was measured in triplicate.

US-EPA 8270 standard mix *N*-nitrosamines were analysed after sample pre-concentration (the procedure is described in Text S1, SI) by GC-MS/MS (Trace 1310 Series GC, Thermo) according to the method described by Chen et al. (2012). LOD and LOQ for the investigated *N*-nitrosamines are reported in Table S1, SI. The measurements were done in duplicate.

Samples obtained from FP tests in ES1 were analysed by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-qTOF). All the details about the analytical method and instruments are reported in Text S2, SI. Data obtained by LC-qTOF analysis were elaborated to verify the presence of a list of proposed compounds, according to the following procedure: the exact mass (m/z) of each compound was extracted from the chromatogram. If a peak with intensity at least 3 orders of magnitude higher than the background noise was observed, the mass spectrum in correspondence of the peak retention time was further analysed. A list of possible molecular formulas was generated by the ChemCalc software (Patinay and Borel, 2013) from the masses observed in the MS spectrum. If the mass of a possible molecular formula corresponded to the theoretical one with an error < 5 ppm, the identification was considered positive.

Samples obtained in FP tests under ES2 were pre-concentrated by solid phase extraction (SPE) following a modified US-EPA Method 521 protocol: 0.5 L samples were passed under vacuum through pre-conditioned Superclean Coconut charcoal 2 g/6 cm^3 cartridges (Supelco), subsequently dried with ultrapure nitrogen gas flow for 15 min. Cartridges were eluted with 6 mL of HPLC grade acetonitrile. Dichloromethane was not used as eluent because this solvent interferes with the signal measured with the nitric oxide analyser. The solvent was evaporated at ambient temperature with a nitrogen flow to a volume of about 2 mL. The recovery of TONO by SPE (η) was specifically assessed for the TONO pools produced by CHD, BZK, CTMA and MET, according to the following procedure: firstly, a 7 days chloramination test was conducted in ES1 (as described in section 2.2) and TONO concentration (C_1) was measured; samples ($V_1 = 10$ mL) were then diluted by ultrapure water to 0.5 L (V_2) of a known TONO concentration (C_2), calculated considering the dilution factor ($C_2 = C_1 \cdot V_1/V_2$). The sample was then concentrated by SPE to about 2 mL (V_3) and the TONO concentration (C_3) was measured. The TONO recovery was finally calculated ($\eta = C_3 \cdot V_3/C_2 \cdot V_2$). The recovery efficiency of SPE was also evaluated for a solution containing 0.16 μM US-EPA 8270 *N*-nitrosamine standard mix, 1 mM phosphate buffer (pH 8) and 2 mM ascorbic acid.

3. Results and discussion

3.1. Total *N*-nitrosamine formation potential (FP)

The results of kinetic tests are shown in Fig. 1, reporting the TONO concentrations as a function of the reaction time and the monochloramine dose for BZK, CTMA, CHD and MET at pH 7. For each NH_2Cl dose, all the selected micropollutants showed a similar trend for the TONO concentrations, rapidly increasing in the first 2–5 days and reaching a stable concentration after about 7 days. For CTMA and MET, a higher NH_2Cl dose yielded a higher TONO formation (TONO concentrations from MET detected only for 100- and 200-fold NH_2Cl molar excess); a similar trend was observed for CHD, for which TONO production was comparable at the highest NH_2Cl doses (100- and 200-fold molar excess). An opposite behaviour was observed for BZK, for which TONO concentrations obtained at 10- and 20-fold molar excess were about 2-times higher than those obtained at 100- and 200-fold molar excess.

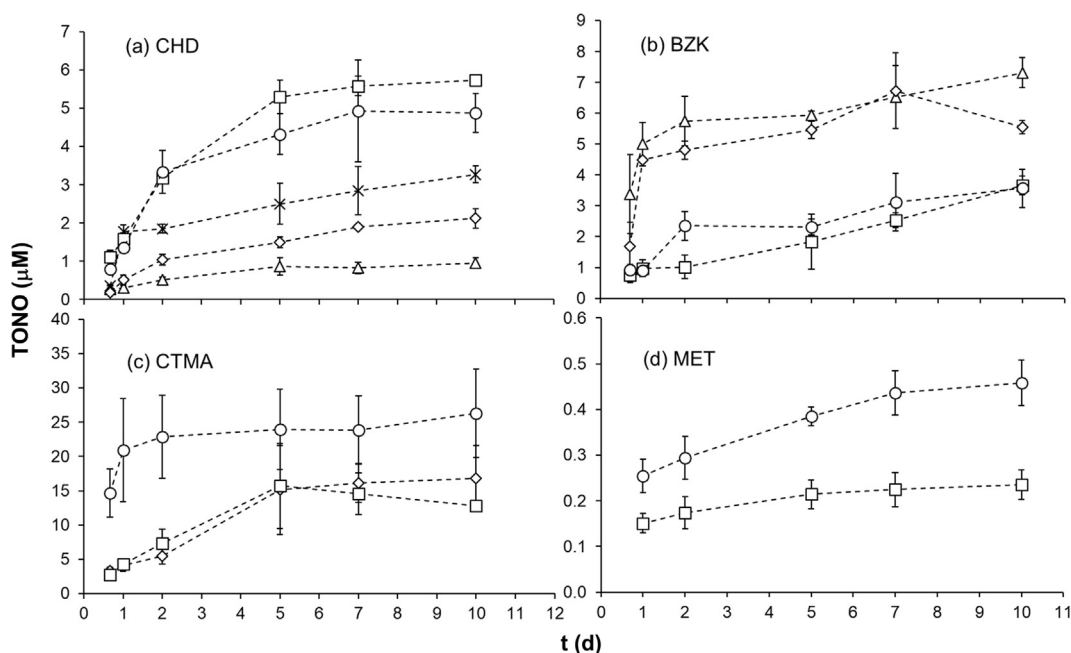


Fig. 1. Kinetics of TONO formation at pH 7 for (a) CHD, (b) BZK, (c) CTMA and (d) MET as a function of the initial molar NH_2Cl excess: 10- (\triangle), 20- (\diamond), 40- (\times), 100- (\square) and 200-fold (\circ) NH_2Cl molar excess. The initial precursor concentration was set at 0.1 mM for CHD and 0.5 mM for the other compounds. Data represent the average \pm standard deviation of three measurement repetitions on two experimental replicates.

This opposing trend for BZK compared to the other precursors is unclear and further studies are needed to elucidate this phenomenon. The monitoring of residual CHD and BZK concentrations during kinetic tests (SI, Text S3 and Figure S2) showed that the precursors were rapidly consumed, with a $>85\%$ abatement after 1 day of reaction. At the same time, the NH_2Cl concentration was expected to decrease over time by disproportionation, as indicated by the monitoring of NH_2Cl in control solutions (SI, Text S3 and Figure S1).

Based on the results of kinetic tests, a 200-fold NH_2Cl molar excess was adopted for FP tests with CHD, MET and CTMA, leading to the maximum TONO formation, while a 10-fold NH_2Cl molar excess was adopted for BZK. For all the precursors, a reaction time of 7 days was selected for FP tests, being sufficient to reach the maximum TONO concentrations.

Samples obtained from FP tests at pH 6, 7 and 8 were firstly analysed for the specific *N*-nitrosamines present in the US-EPA 8270 standard mix. Among the analysed samples, NDMA concentrations higher than the LOQ ($0.09 \mu\text{M}$), corresponding to molar yields $>0.09\%$ for CHD and $>0.02\%$ for the other precursors, were detected only for BZK at pH 8, while in all the other samples NDMA was not detectable. None of the other *N*-nitrosamines of the US-EPA 8270 standard mix were detected after the FP tests for any of the precursors.

The average NDMA concentration formed by BZK was $2.81 \pm 0.13 \mu\text{M}$, corresponding to a molar formation yield of $0.56 \pm 0.03\%$ (Table 2). This value is consistent with previous observations (Kemper et al., 2010) at pH 7 for the two main constituents of the benzalkonium chloride mixture: benzyldimethyldodecylamine (0.28% molar NDMA yield) and benzyldimethyltetradecylamine (0.26% molar NDMA yield).

No NDMA formation was observed for CHD, CTMA and MET (NDMA concentrations $< \text{LOD}$). While, to our knowledge, NDMA formation from CHD has never been reported, the results obtained for CTMA and MET were not unexpected, based on previous findings. In fact, relatively low molar NDMA yields for CTMA (0.03%)

and MET ($<0.04\%$) have been observed in previous studies (Kemper et al., 2010; Shen and Andrews, 2011a). Hence, considering these low molar yields, NDMA concentrations close to the LOQ would have been expected in the current study and were therefore not detected.

The determined TONO FP for CHD, MET, BZK and CTMA at various pH values are reported in Table 2, expressed as molar TONO yields. Unlike the US-EPA 8270 standard mix for *N*-nitrosamines, TONO measurements displayed detectable *N*-nitrosamine concentrations in all the samples. The TONO formation increased with increasing pH for all precursor compounds. The pH-dependence of the *N*-nitrosamine formation can be explained by (i) the increasing NH_2Cl stability at higher pH and (ii) the acid-base speciation of some of the precursors. The NH_2Cl self-decomposition (SI, Figure S1) is slower at higher pH values, resulting in a higher NH_2Cl exposure during the FP test, which in turn may lead to a higher total *N*-nitrosamine production. For CHD and MET, the increase in TONO formation with increasing pH can be explained by the acid-base speciation of these compounds (pK_a -values in Table 1). The fractions of the deprotonated forms of CHD and MET (as well as TMA, DMBA and DMBzA) increase with increasing pH, which enhances the nucleophilic attack on NH_2Cl , resulting in significantly higher *N*-nitrosamine yields (Selbes et al., 2013).

Among the studied precursor compounds, CHD exhibited the highest molar TONO yields at all pH values, ranging from $2.64 \pm 1.45\%$ at pH 6 to $11.92 \pm 0.83\%$ at pH 8. Molar TONO yields from $1.15 \pm 0.09\%$ (pH 6) to $4.93 \pm 1.42\%$ (pH 8) were obtained for CTMA, while BZK showed molar conversions of between $0.68 \pm 0.24\%$ and $2.12 \pm 0.40\%$, depending on the pH. Among the studied micropollutants, MET showed the lowest TONO yields ($\leq 0.21 \pm 0.03\%$), being at least one order of magnitude lower than for the other compounds, for a given pH value.

These results demonstrate the relatively low NDMA formation yields of the studied micropollutants, consistent with the results recently reported by Woods-Chabane et al. (2017) for tertiary and quaternary amines in chloraminated wastewater samples.

Table 2

Molar yields of NDMA and TONO observed in formation potential (FP) experiments under experimental setup ES1 (reaction time: 7 days; initial precursor concentration: 0.1 mM for CHD, 0.5 mM for the other compounds; initial NH_2Cl molar excess: 10-fold for BZK, 200-fold for the other compounds) and ES2 (reaction time: 7 days; initial precursor concentration: 0.5 μM ; initial NH_2Cl molar excess: 200-fold). Reported molar yields are the average values of multiple measurement repetitions (two for NDMA and three for TONO) on two experimental replicates.

Precursor	pH	Molar yield (%)		
		NDMA ^a		TONO ^b
		average (range)	average \pm st.dev.	
		ES1	ES1	ES2
Micropollutants				
Chlorhexidine hydrochloride	6	<0.09	2.64 \pm 1.45	-
	7	<0.09	4.93 \pm 1.33	-
	8	<0.09	11.92 \pm 0.83	6.54 \pm 0.39
Metformin dihydrochloride	6	<0.09	0.04 \pm 0.003	-
	7	<0.09	0.09 \pm 0.01	-
	8	<0.09	0.21 \pm 0.03	0.77 \pm 0.32
Benzalkonium chloride	6	<0.09	0.68 \pm 0.24	-
	7	<0.09	1.31 \pm 0.21	-
	8	0.56 (0.55–0.56)	2.12 \pm 0.40	4.05 \pm 0.38
Cetyl trimethyl ammonium chloride	6	<0.09	1.15 \pm 0.09	-
	7	<0.09	4.77 \pm 1.00	-
	8	<0.09	4.93 \pm 1.43	4.05 \pm 0.01
Model compounds				
Tetramethyl ammonium chloride	6	<0.09	0.04 \pm 0.002	-
	7	<0.09	0.07 \pm 0.004	-
	8	<0.09	0.90 \pm 0.08	-
Trimethyl amine	6	<0.09	<1.0	-
	7	<0.09	0.03 \pm 0.005	-
	8	0.10 ^c	0.29 \pm 0.007	-
<i>N,N</i> -dimethylbutyl amine	6	<0.09	<1.0	-
	7	<0.09	0.02 \pm 0.004	-
	8	0.05 ^c	0.17 \pm 0.03	-
<i>N,N</i> -dimethylbenzyl amine	6	<0.09	<1.0	-
	7	1.22 (1.08–1.36)	0.72 \pm 0.03	-
	8	5.05 (3.92–6.18)	3.27 \pm 0.34	-

^a LOQ for NDMA: 0.09 \pm 0.001 μM .

^b LOQ for TONO: 1.0 \pm 0.45 μM .

^c NDMA concentration > LOQ only in one sample.

However, despite the low NDMA formation yield, the studied precursors can produce significant TONO concentrations. The composition of the formed TONO is unknown and is further discussed in section 3.3. Even though the overall mutagenicity potential of the TONO pool cannot be easily evaluated, the molar TONO yields suggest that the corresponding precursors should be taken into consideration during chloramination of wastewaters and drinking waters. Being associated to consumer products, the studied precursors can easily enter the aquatic system by wastewater discharge and get into the drinking water by this way. For example, BZK homologues have been detected in wastewater effluents, river waters and groundwaters at concentrations ranging from 10 ng L^{-1} to 65 $\mu\text{g L}^{-1}$ (Clara et al., 2007; Ding and Liao, 2001; Estévez et al., 2012; Ferrer and Furlong, 2001; Martínez-Carballo et al., 2007). Relevant concentrations ($\leq 0.66 \mu\text{g L}^{-1}$) of alkyl-trimethylammonium chloride compounds, including CTMA, were found in Taiwanese rivers (Ding and Tsai, 2003), while concentrations in the range 10–150 ng L^{-1} were detected in Austrian surface waters (Martínez-Carballo et al., 2007). CHD concentrations of up to 5 ng L^{-1} have been found in a drinking water reservoirs in Turkey (Yavuz et al., 2015), while concentrations between 46.1 and 78.1 ng L^{-1} were detected in a Spanish DWTP influent treating river water (Boleda et al., 2011). Scheurer et al. (2012) detected MET concentrations between 18 and 105 $\mu\text{g L}^{-1}$ in five German WWTP influents and estimated a discharge in recipient rivers between 8 and 700 g d^{-1} , due to their incomplete elimination during

wastewater treatment. The same authors found MET concentrations between 0.06 and 2.1 $\mu\text{g L}^{-1}$ in eight German rivers. Considering the highest concentrations reported in the literature for surface waters or DWTP intakes and applying the maximum TONO molar yields determined in this study (at pH 8), TONO FP of the selected precursors could vary from 0.02 nM (1.2 ng L^{-1} expressed as NDMA) to 3.97 nM (293.5 ng L^{-1} expressed as NDMA), for CHD and BZK, respectively. Although intake concentrations are strongly case specific, these estimates indicate that the contribution of the studied precursors could be relevant. The determined TONO FPs are consistent with those previously observed by Dai and Mitch (2013) in drinking water systems, with measured TONO concentrations in the same order of magnitude (1 nM median concentration). An additional important factor to be considered for evaluating the actual exposure to *N*-nitrosamine precursors is their fate in aquatic systems, leading to a natural attenuation of their concentrations at DWTP intakes. For instance, QACs are known to be easily adsorbed onto negatively charged solids, such as soils and sludge, and to have a relatively low biodegradability, especially when containing benzyl or alkyl functional groups (García et al., 1999; Ikehata and El-Din, 2004; Li and Brownawell, 2010; Ying, 2006). In contrast to a low *N*-nitrosamine molar yield, MET is characterized by high mobility in natural aquatic systems and can be easily distributed in the aquatic environment, due to the high persistence and low hydrophobicity ($\log K_{OW} = -2.64$) (Lindim et al., 2017; Mansour et al., 2016; Trautwein et al., 2014). Even if the precursors are partially

metabolized, they can contribute to *N*-nitrosamine formation (Shen and Andrews, 2011a). For instance, typical biodegradation products of QACs are tertiary and secondary amines, which can lead to *N*-nitrosamine formation yields much higher than their parent compounds (Ying, 2006; Li and Brownawell, 2010).

3.2. Total *N*-nitrosamine formation potential of model tertiary and quaternary amines

The *N*-nitrosamine formation potential during chloramination was also determined for various tertiary and one quaternary amine model compounds (Table 1): trimethyl amine (TMA), *N,N*-dimethylbutyl amine (DMBA), *N,N*-dimethylbenzyl amine (DMBzA) and tetramethyl ammonium (TeMA). Molar NDMA and TONO yields obtained in the pH range 6–8 are reported in Table 2. Similar to the selected micropollutants, for these model compounds, NDMA was the only detectable compound from the US-EPA 8270 standard *N*-nitrosamines mix. Quantifiable NDMA and TONO concentrations were found for all the studied model tertiary amines (TMA, DMBA and DMBzA). TMA displayed TONO concentrations at pH 7 slightly higher than the LOQ, resulting in a molar conversion yield of 0.03%. A NDMA concentration higher than the LOQ was determined only at pH 8, with an average molar yield of 0.10%, while TONO concentrations corresponded to a molar yield of 0.29%. The TONO FP for TMA at pH 8 was higher than the NDMA FP, which is probably due to the accuracy of the two analytical methods and related mainly to the different sample pre-treatment procedures. In particular, GC-MS/MS analysis could be affected by a significant loss of NDMA due to the sample extraction procedure, which was not necessary for TONO analysis. A formation of non-NDMA *N*-nitrosamines is not possible from TMA. Furthermore, the TONO method applied in this study (Breider and von Gunten, 2017) tends to slightly overestimate NDMA concentrations ($115\% \pm 5\%$ recovery of NDMA from standard curve of US-EPA 8270 *N*-nitrosamines standard mix), which may be an additional explanation for the observed discrepancy.

NDMA was not detected in DMBA samples at pH 6 and 7, while TONO concentrations slightly above the LOQ (corresponding to 0.02% molar yield) were measured at pH 7. NDMA and TONO molar yields of 0.05% and 0.17%, respectively, were obtained at pH 8. Similar to TMA, the NDMA FP observed for DMBA at pH 8 was lower than the TONO FP and the difference in NDMA and TONO concentrations can again be mainly attributed to the differences in the analytical methods.

Among the model tertiary amines, DMBzA displayed the highest *N*-nitrosamine formation potentials. Molar NDMA yields were, on average, 1.22% at pH 7 and 5.05% at pH 8, while molar TONO yields were 0.72% or 3.27% at pH 7 or 8, respectively. The NDMA FPs resulted to be higher than TONO FPs in this case. However, considering the uncertainties associated with the two analytical methods, measured NDMA and TONO concentrations can be considered to be comparable, indicating that NDMA was the most prevalent *N*-nitrosamine present in the analysed samples. The relatively high formation of NDMA from DMBzA can be attributed to the low stability of the benzyl group, which is easily released from the N-atom (Kemper et al., 2010; Selbes et al., 2013; Spahr et al., 2016). In fact, the benzyl group is a weaker base compared to alkyl groups and plays a stronger electron donating effect on the DMA moiety, resulting in higher NDMA formation rates (Shen and Andrews, 2011a).

Comparing the molar yields of NDMA and TONO for all the selected tertiary amines (MET, TMA, DMBA and DMBzA), the role of the molecular structures of the precursors, in particular the type of the functional group bound to the DMA moiety, becomes evident. Even though it was not possible to evaluate the TONO composition,

experimental results confirmed that the benzyl functional group is much more easily released than the methyl, butyl or biguanide moieties, leading to significantly higher NDMA yields.

For TeMA, the only tested quaternary amine model compound, NDMA concentrations higher than the LOD were never detected, while quantifiable TONO FPs were found at all the pHs. TONO concentrations at pH 6 and 7 were slightly higher than the LOQ, while quantifiable concentrations were measured at pH 8, corresponding to a 0.90% molar TONO yield. Since NDMA is expected to be the most prevalent *N*-nitrosamine formed from TeMA, the significant difference between NDMA and TONO yields observed at pH 8 could be explained by an underestimation of NDMA concentration due to the sample extraction procedure. Even though the TONO composition observed for TeMA was not characterized, the results obtained for other precursors show a link between the molecular structures of QACs and *N*-nitrosamine formation. The TONO FP for TeMA was much lower than for the QACs BZK and CTMA, with a significant difference in molar yields (difference >60%, considering the average molar yields reported in Table 2 for pH 8). This indicates that the functional groups bound to the N-atom of QACs play an important role for the *N*-nitrosamine conversion efficiency.

A previous study showed that formation of specific *N*-nitrosamines (i.e. NDMA and NDBA) from chloramination of QACs is mostly associated to QACs themselves rather than lower order amine impurities (Kemper et al., 2010). However, the negligible contribution of tertiary amine impurities has not been demonstrated for the TONO FP. The selected tertiary amine model compounds in the current study contain the same, or very similar, functional groups as potential impurities in the QACs. Although the aliphatic chain is shorter in DMBA compared to BZK and CTMA, previous studies have shown that the length of the alkyl group next to the nitrogen atom does not significantly affect the *N*-nitrosamine conversion yield (Selbes et al., 2013). Results of FP tests showed that molar *N*-nitrosamine yields of DMBA and TMA were never high enough to explain *N*-nitrosamine formation from QACs by the presence of impurities (maximum estimated contribution to NDMA or TONO FPs of BZK and CTMA <1%). DMBzA, for which the highest NDMA FP was observed, could contribute up to the 45% of NDMA formation potential of BZK, if considering a maximum content of 5% as impurity (BZK purity >95%). In contrast, the maximum contribution of DMBzA impurities to the TONO FP of BZK would be $\leq 8\%$, indicating that most of the uncharacterized *N*-nitrosamine production is due to BZK itself. Hence, the results from this study indicate that the observed TONO FP from QACs cannot be attributed to tertiary amine impurities and that QACs themselves are the dominant *N*-nitrosamine precursors.

3.3. Elucidation of the potential structures of unknown *N*-nitrosamines formed from quaternary ammonium compounds

TONO constituents formed from the selected consumer product ingredients were partially characterized only for BZK, for which it was possible to determine the NDMA FP. The ratio between the average observed molar concentrations of NDMA and TONO was 0.26. Even by taking the differences in the two analytical methods into account, the difference between NDMA and TONO concentrations is significant. This indicates that a large fraction of the total *N*-nitrosamine pool consists of unknown compounds. So far, no known *N*-nitrosamine has been identified in the TONO pools formed from CHD, MET and CTMA.

Because no compounds from the US-EPA 8270 standard *N*-nitrosamines mix were found, some other *N*-nitrosamines must be responsible for the measured TONO. Possible *N*-nitrosamine structures formed by CHD cannot be easily proposed due to the

complex molecular structure of the precursor. In contrast to most of the tertiary or quaternary amines studied in the literature, CHD does not contain a dialkylamino group which can be related to the formation of the corresponding *N*-nitrosamine, such as NDMA, NDEA or NDBA (Kemper et al., 2010; Le Roux et al., 2011; Selbes et al., 2013; Shen and Andrews, 2011a; Spahr et al., 2016). MET contains similar biguanide moieties as CHD (Table 1), however, it showed significantly lower *N*-nitrosamine FP. Comparing the molecular structures of the two precursors, the higher *N*-nitrosamine formation yield of CHD can be attributed to the aromatic moiety, which is bound to the end-membered N-atom of the biguanide group. In comparison, MET contains the same biguanide structure, however, with an end membered DMA moiety. The low NDMA FP of MET has been explained by the electron-withdrawing effect of the biguanide group bound to DMA leading to a lower nucleophilicity of the N-atom (Le Roux et al., 2011; Selbes et al., 2013; Shen and Andrews, 2011a). In contrast, the chlorinated aromatic ring in the CHD structure could enhance the nucleophilic attack on the chloramine. Nevertheless, further studies are needed to verify these assumptions.

For QACs, the presence of only one N-atom limits the number of possible reactions with NH_2Cl leading to potential *N*-nitrosamine products. It has been demonstrated that *N*-nitrosamine formation from tertiary and quaternary amines involves the release of the functional groups bound to the nitrogen atom in the precursor molecule (Selbes et al., 2013). Various studies have shown that the structure of the leaving groups in precursors containing a DMA moiety strongly influences the formation of NDMA, resulting in a large range of formation yields (Kemper et al., 2010; Le Roux et al., 2011; Selbes et al., 2013; Shen and Andrews, 2011b). However, most of these studies focused on NDMA formation and TONO

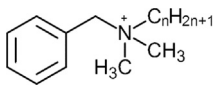
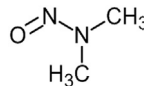
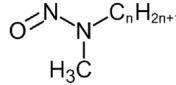
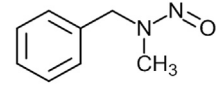
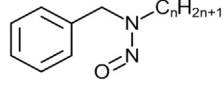
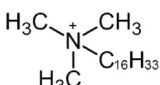
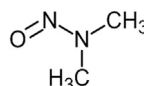
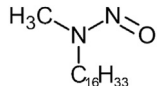
measurements were rarely performed.

In the current study, the structures of other potential *N*-nitrosamines were hypothesized based on all possible leaving groups present in the precursor molecule. The potential *N*-nitrosamines resulting from chloramination of BZK and CTMA are shown in Table 3. For instance, the benzyl group and the aliphatic chain ($\text{C}_n\text{H}_{2n+1}$) bound to the N-atom in BZK are supposed to act as leaving groups during NDMA formation. Similarly, the release of one methyl group and the 16-C aliphatic chain ($\text{C}_{16}\text{H}_{33}$) may lead to the formation of NDMA from CTMA. The other *N*-nitrosamine structures were proposed by considering all the other potential leaving groups, including the methyl group, the benzyl group and the long-chain alkyl group for BZK, the methyl groups and the 16-C aliphatic chain for CTMA.

To test the formation of the proposed *N*-nitrosamines, the samples obtained by the TONO FP tests at pH 8 were analysed by high resolution LC-qTOF. The high accuracy and resolution of the obtained mass spectra allow proposing a compound based on its exact mass, in absence of analytical reference standards. The procedure described in section 2.3 was applied to screen the list of potential *N*-nitrosamines from the chromatograms, by extracting their exact masses (m/z) (calculated by the ChemCalc software and reported in Table 3, including the protonated molecules). The procedure was also applied to detect potential denitrosation products of the investigated compounds.

Among the hypothesized *N*-nitrosamines, a positive match was obtained for BZK. The full chromatogram of the BZK sample (treated with 10-fold molar excess of monochloramine at pH 8) is reported in Figure S3, SI. The extraction of the exact mass of $\text{C}_{13}\text{H}_{28}\text{N}_2\text{O}$ ($[\text{M}+\text{H}]^+ = 229.2279$) allowed to obtain a distinct peak (intensity of $8.56 \cdot 10^5$ arbitrary units) at a retention time of

Table 3
Structure, formulas and exact masses of the hypothetical *N*-nitrosamines formed from BZK and CTMA. Only *N*-nitroso-*N*-methyldodecylamine could be confirmed as a product from chloramination of BZK.

Precursor	Hypothesized <i>N</i> -nitrosamines		Exact mass (m/z)	
	Structure	Formula	$[\text{M}]^+$	$[\text{M}+\text{H}]^+$
BZK  $n=8, 10, 12, 14, 16, 18$		$\text{C}_2\text{H}_6\text{N}_2\text{O}$	74.0480	75.0558
		$\text{C}_{n+1}\text{H}_{2n+4}\text{N}_2\text{O}$ $n=8, 10, 12, 14, 16, 18$	172.1576 200.1888 228.2202 256.2515 284.2828 312.3141	173.1654 201.1967 229.2279 257.2593 285.2906 313.3219
	$n=8, 10, 12, 14, 16, 18$		150.0793	151.0871
		$\text{C}_8\text{H}_{10}\text{N}_2\text{O}$		
		$\text{C}_{n+7}\text{H}_{2n+8}\text{N}_2\text{O}$ $n=8, 10, 12, 14, 16, 18$	248.1888 276.2202 304.2515 332.2828 360.3141 388.3454	249.1967 277.2280 305.2593 333.2906 361.3219 389.3532
CTMA 		$\text{C}_2\text{H}_6\text{N}_2\text{O}$	74.0480	75.0558
		$\text{C}_{17}\text{H}_{36}\text{N}_2\text{O}$	284.2828	285.2906

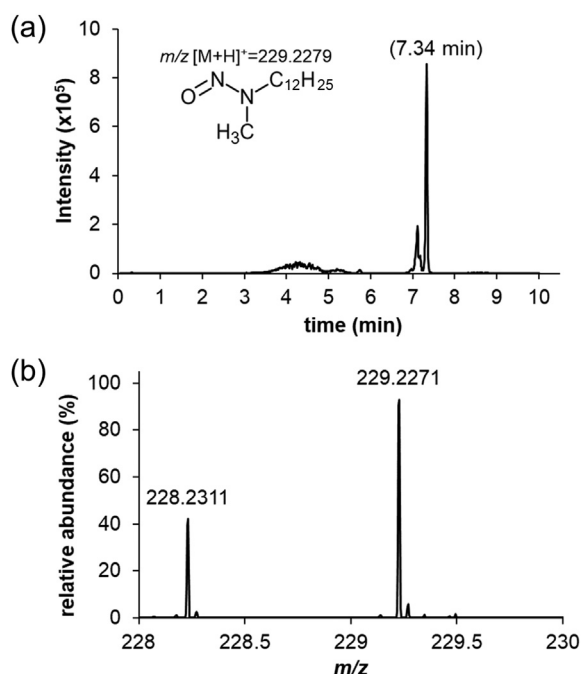


Fig. 2. High resolution LC-qTOF results for a BZK sample (FP test at pH 8): (a) chromatogram obtained by extraction of the exact mass of $C_{13}H_{28}N_2O$ ($m/z [M+H]^+ = 229.2279$); (b) mass spectrum at retention time = 7.34 min.

7.34 min (Fig. 2 a). The relative mass spectrum (Fig. 2 b) showed a peak at $m/z = 229.2271$, corresponding to a relative abundance of 93% and matching with high accuracy (error < 0.1%) with two possible molecular formulas: (i) the hypothesized *N*-nitrosamine ($C_{13}H_{28}N_2O$, error = 3.876 ppm, Table 3) and (ii) $C_{11}H_{27}N_5$ (error = 1.981 ppm). The formation of the latter compound is highly improbable considering the investigated reaction system. The identified mass corresponded with a high level of confidence to *N*-nitroso-*N*-methyldodecylamine (Fig. 2 a), a potential product of the reaction of chloramine with benzyldimethyldodecylamine, which is the most abundant constituent of the benzalkonium chloride mixture (about 70% abundance, according to the supplier (Sigma-Aldrich)).

The detection of *N*-nitroso-*N*-methyldodecylamine was not unexpected and its formation at significant yield is consistent with previous results reported in the literature (Kemper et al., 2010; Selbes et al., 2013; Spahr et al., 2016). Because the benzyl group is an electron-donating group, which increases the electron density on the N-atom, a nucleophilic attack on the chloramine is favoured. In addition, benzyl-type functional groups are known to be better leaving groups than alkyl groups, both in tertiary and quaternary amine precursors (Kemper et al., 2010; Selbes et al., 2013). Hence, during chloramination of BZK homologues, *N*-nitroso-*N*-methylalkylamines are expected to have higher yields than benzyl-containing *N*-nitrosamines. Consistently, it was found previously that the molar yield of *N*-nitrosomethylbenzylamine from benzyldimethyltetradecylamine (the C-14 constituent of BZK) was only 0.016%, about 16-times lower than the molar NDMA yield (Kemper et al., 2010).

High resolution LC-qTOF analysis of a CTMA sample did not lead to an identification of the hypothesized compound ($C_{17}H_{36}N_2O$) with enough confidence (results are reported in Figures S3 and S4, SI). The formation of a compound during FP tests cannot be excluded from the absence of a positive match. It may be caused by artefacts in the detection method, e.g., the stability of the *N*-nitroso group during ionization. Moreover, the absence of reference

standards did not allow determining an optimal sample extraction procedure and optimized analytical conditions for each proposed *N*-nitrosamine. Thus, the performed analyses must be considered as a first attempt to characterize the unknown fraction of TONO. Nevertheless, in the current study, in addition to NDMA, a novel *N*-nitrosamine from the TONO FP pool of BZK was potentially identified. This finding strongly supports the hypothesis that different leaving groups in the precursor molecule can lead to the formation of different *N*-nitrosamines.

The novel *N*-nitrosamine, *N*-nitroso-*N*-methyldodecylamine was found to be carcinogenic in experimental animal tests and its presence in personal care and household products has already been cause of concern in previous studies (Hecht et al., 1982; Kamp and Eisenbrand, 1991; Lijinsky et al., 1983; Morrison and Hecht, 1982; SCCS, 2012). For instance, a T25 of $0.46 \text{ mg kg}^{-1} \text{ d}^{-1}$ (being the chronic dose rate giving tumours in the 25% of the tested animals (Dybing et al., 1997)) was determined in laboratory rats for *N*-nitroso-*N*-methyldodecylamine, which is comparable to the values obtained for other *N*-nitrosamines of the US-EPA 8270 standard mix (varying from $0.058 \text{ mg kg}^{-1} \text{ d}^{-1}$ of NDMA to $0.57 \text{ mg kg}^{-1} \text{ d}^{-1}$ of NPYR) (SCCS, 2012). Hence, it may significantly contribute to the overall cancer potency of the total *N*-nitrosamine pool. Even though the high-resolution LC-qTOF results were not quantitative, they indicate that the uncharacterized fraction of TONO can be composed of species as hazardous as the compounds from the US-EPA 8270 standard *N*-nitrosamine mix. Even if a full characterization of the TONO pool is not always possible, coupling total and specific (i.e., US-EPA 8270 standard mix) *N*-nitrosamine analyses is an effective way to fully assess the potential hazardousness of *N*-nitrosamine precursors. Product identification in the TONO FP pool by LC-qTOF may lead to further insights into the formation of individual compounds, which might be of toxicological concern.

3.4. Effect of precursors concentration on TONO formation potential

Experimental conditions adopted in ES1 were suitable for determination of the *N*-nitrosamine FP of the studied precursors and investigating the composition of the formed TONO compound pool. However, the precursor and the monochloramine concentrations were much higher compared to realistic systems, leading to the formation of extremely high *N*-nitrosamine concentrations. To validate the molar TONO yield values obtained in ES1, FP tests were also conducted in ES2, with experimental conditions closer to real water matrices (pH 8, $0.5 \mu\text{M}$ precursor and $100 \mu\text{M}$ monochloramine, corresponding to 5.1 mg L^{-1} as NH_2Cl). The relatively long reaction time in FP tests (7 days) was mainly selected to maximize *N*-nitrosamine formation and can be considered as an upper limit of residence times in drinking water distribution systems. In contrast, it is longer than typical contact times for wastewater disinfection. Due to the lower *N*-nitrosamine concentrations produced under ES2, TONO concentrations were measured after sample pre-concentration by solid phase extraction (SPE).

As discussed above, the composition of the generated TONO compound pool is different for each precursor. Since the physico-chemical properties of the formed *N*-nitrosamines can strongly affect the efficiency of SPE (Dai and Mitch, 2013), the recovery of TONO concentrations by SPE was determined for the specific *N*-nitrosamine pools formed by each precursor, as reported in section 2.2. The TONO recovery efficiencies (values reported in Table S2, SI) resulted to be quite low for all the precursors, with values of about 10%. Applying the obtained recovery efficiency values to the TONO concentrations measured in the FP tests (ES2), the molar TONO yields resulted, on average as $6.54 \pm 0.39\%$, $4.05 \pm 0.38\%$, $4.05 \pm 0.02\%$ and $0.77 \pm 0.32\%$ for CHD, BZK, CTMA and MET, respectively. Despite the extremely different experimental

conditions and the uncertainty associated to SPE, the molar TONO yields obtained under ES2 were comparable with those obtained in ES1 at pH 8 (Table 2). CHD and MET displayed the highest and the lowest molar TONO yields, respectively, while intermediate values were obtained for QACs. Even though the molar TONO yields are slightly different, they are in the same order of magnitude for ES1 and ES2. This indicates that results obtained in ES1 can be considered representative for more realistic conditions.

When dealing with TONO measurements in real water matrices, the recovery efficiency of SPE plays a key role and it can strongly vary with the TONO pool composition (Dai et al., 2015; Zeng and Mitch, 2015). In this study, the SPE extraction efficiency was also evaluated for a 0.16 μ M US-EPA 8270 standard *N*-nitrosamine mix solution, both in terms of TONO and specific *N*-nitrosamine constituents. Some specific *N*-nitrosamines (such as NMOR, NDEA and NDBA) were not detected after SPE, denoting a very low recovery. Nevertheless, the average recovery efficiency of the TONO concentrations was 42%. The variability in the recovery efficiencies obtained for samples from FP tests demonstrates that the studied precursors lead to the formation of quite a large range of *N*-nitrosamines, having different affinities to the SPE material and probably yielding higher analytical uncertainty. The relatively low recovery of TONO concentration observed for the reacted samples could be due to the presence of unreacted precursor molecules, having surface active properties and being present at high initial concentrations, which could form micellar aggregates with the *N*-nitrosamines present in solution and increase their hydrophilicity. Further studies are needed to elucidate the composition and the properties of the observed TONO pools and to optimize the SPE procedure.

4. Conclusions

Specific and total *N*-nitrosamine (TONO) formation potentials (FP) for different micropollutants (chlorhexidine, CHD, metformin, MET, benzalkonium chloride, BZK, and cetyltrimethylammonium chloride, CTMA) and model compounds (trimethylamine, TMA, *N,N*-dimethylbutylamine, DMBA, *N,N*-dimethylbenzylamine, DMBzA, and tetramethylammonium, TeMA) were determined at pH 6, 7 and 8. All the micropollutants displayed quantifiable molar TONO yields, with maximum values at pH 8 varying between 0.21% (MET) and 11.92% (CHD). A quantifiable NDMA FP was determined only for BZK, with a molar yield of 0.56% (at pH 8) and corresponding to about 26% of the TONO FP. Generally, the formed TONO compounds consisted mostly of uncharacterized species, not included in the US-EPA 8270 *N*-nitrosamines standard mix.

The study of tertiary and quaternary model amines revealed a role of precursor molecular structures, in particular the nature of functional groups, in NDMA and TONO conversion yields: DMBzA displayed the highest molar NDMA yields, indicating that the presence of a benzyl functional group in tertiary amines containing a DMA moiety results in significantly higher NDMA formation. BZK and CTMA showed molar TONO yields much higher than TeMA, suggesting that the formation of uncharacterized *N*-nitrosamines from QACs is favoured by the presence of benzyl and long-chain alkyl functional groups, instead of only methyl groups.

A screening procedure with high resolution LC-qTOF analysis allowed to identify with high probability an uncharacterized *N*-nitrosamine (*N*-nitroso-*N*-methyl dodecylamine) from the chloramination of BZK. The detection of a second *N*-nitrosamine species in addition to NDMA points out that different functional groups in QAC molecules can act as leaving groups during chloramination and form different *N*-nitrosamine structures at significant yield. These results represent a first step in the understanding of *N*-nitrosamine formation during chloramination of QACs.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.watres.2018.02.019>

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