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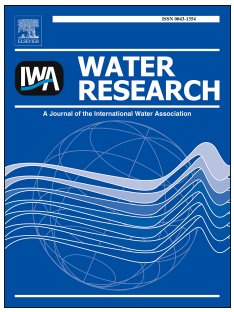

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Specific and total *N*-nitrosamines formation potentials of nitrogenous micropollutants during chloramination

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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*-methyldodecylamine) 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.

Keywords: total *N*-nitrosamines (TONO), NDMA, nitrogenous micropollutants, *N*-nitrosamines formation potential, chloramination

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, 2016).

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*-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

92 purification. This indicates that the QAC itself, rather than impurities, was the dominant *N*-nitrosamine
93 precursor. Even though the reaction pathways have not been defined, the same authors suggested that
94 the NDMA formation from QACs may involve amidogen or chloramino radicals formed from
95 chloramines. Some quaternary amine monomers (e.g., choline, cocoamidopropyl betaine) and
96 polymers (e.g., polyDADMAC, polyquaternium-7, polyacrylamide, polyamine and DMA-epi-DMA) were
97 also found to form NDMA during ozonation (Marti et al., 2015; Padhye et al., 2011), free chlorine and
98 chlorine dioxide disinfection (Park et al., 2009a; Zhang et al., 2014), even though at molar yields lower
99 than those observed for chloramination.
100 The majority of the studies in the literature deals with the formation of NDMA, being the most often
101 detected *N*-nitrosamine in tap and wastewater (Russell et al., 2012). However, previous studies
102 showed that NDMA is often a minor part of the total *N*-nitrosamines (TONO) pool (Dai and Mitch,
103 2013; Kulshrestha et al., 2010; Zeng and Mitch, 2015). Analysing 36 drinking water treatment plants
104 and distribution systems, Dai and Mitch (2013) found that NDMA made up only 5% of the TONO pool
105 on a median basis. Zeng and Mitch (2015) observed that the contribution of NDMA to TONO was
106 1-11% and 3-60% in domestic greywater streams treated by chloramine and ozone, respectively. The
107 same authors suggested that the precursors of the uncharacterized *N*-nitrosamine fraction were
108 associated to common macro-constituents of consumer products, such as QACs present in shampoos
109 and soaps.
110 While many NDMA precursors were identified in the literature, the determination of the TONO
111 formation potential (FP) of specific model compounds is not a common procedure. The investigations
112 of *N*-nitrosamine formation are typically limited to the species detected by the US-EPA method 521
113 (US EPA, 2005), while the identity of the other components of the TONO pool remains unclear.
114 The goal of this study was to investigate the formation of *N*-nitrosamines during chloramination of
115 various nitrogenous compounds that can be discharged to municipal wastewater and may end up in
116 the receiving water bodies. The investigated compounds included two quaternary ammonium
117 compounds used as disinfectants and surfactants (benzalkonium chloride, BZK, a mixture of benzyl
118 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 H_2O), 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 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 $\mu g\,mL^{-1}$ 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 (NH_2Cl) and dichloramine ($NHCl_2$) (see section 2.3) in the stock solutions were measured after preparation; the $NHCl_2$ 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 NH_2Cl concentration over time (see section 2.3) in buffered ultrapure water solutions. 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

174 *N*-nitrosamine production (according to kinetic test results discussed in section 3.1). The
175 concentrations of TONO and *N*-nitrosamines of the US-EPA 8270 standard mix (see section 2.3) were
176 measured at the end of the FP tests.
177 In ES2, 7 days FP tests were conducted in 0.5 L solutions, containing deionized water, 1 mM sodium
178 phosphate buffer (pH 8), 100 μM and 0.5 μM initial concentrations of monochloramine and precursor,
179 respectively (corresponding to a 200-fold NH_2Cl molar excess). The TONO concentration was
180 measured after sample pre-concentration by solid phase extraction (see section 2.3).
181 The molar yield of *N*-nitrosamines formation was calculated as the ratio between the produced molar
182 concentration of the *N*-nitrosamine and the molar concentration of the precursor.
183 For all the chloramination experiments, reaction solutions were quenched at the end of the
184 pre-determined reaction time by dosing an ascorbic acid stock solution (280 mM) to get a
185 stoichiometric excess relative to the initial NH_2Cl concentration. Quenched solutions were stored at
186 4°C in the dark. All the experiments were performed in duplicate.
187

188 **2.3 Analytical methods**

189 NH_2Cl and $NHCl_2$ concentrations were determined spectrophotometrically (Shimadzu, UV-1800) using
190 their respective molar extinction coefficients at 245 and 295 nm, respectively and solving the
191 equations expressing absorbance values at the two wavelengths simultaneously (Schreiber and Mitch,
192 2005). The $NHCl_2$ concentration is quantifiable only when the ratio between absorbance values at
193 295 nm and 245 nm is ≥3%, corresponding to a $NHCl_2$:NH_2Cl molar ratio ≤0.01%.
194 Total *N*-nitrosamine concentrations were measured by a method based on UV photolysis and
195 chemiluminescence measurement of nitric oxide (see Breider and von Gunten (2017) for more
196 details). The chemiluminescence signal was related to the TONO concentration by a standard curve,
197 obtained with different concentrations of the US-EPA 8270 *N*-nitrosamine standard mix (from 0.8 to
198 16.2 μM of each *N*-nitrosamine). The test solutions in absence of monochloramine were compared
199 with ultrapure water, to verify the absence of a background signal. The calibration was repeated daily
200 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 (LOD=3.3×SE/m, LOQ=10×SE/m). Average (±standard deviation) LOD and LOQ resulted equal to 0.3±0.15 μM and 1.0±0.45 μM, 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 (Patiny 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 2g/6cm^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 ($\eta=C_3 \cdot V_3/C_2/V_2$). 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 Figure 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. 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 μM), 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±0.13 μM, corresponding to a molar formation yield of 0.56±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 <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

281 increasing pH can be explained by the acid-base speciation of these compounds (pK_a-values in Table
282 1). The fractions of the deprotonated forms of CHD and MET (as well as TMA, DMBA and DMBzA)
283 increase with increasing pH, which enhances the nucleophilic attack on NH_2Cl, resulting in significantly
284 higher *N*-nitrosamine yields (Selbes et al., 2013).

285 Among the studied precursor compounds, CHD exhibited the highest molar TONO yields at all pH
286 values, ranging from 2.64±1.45% at pH 6 to 11.92±0.83% at pH 8. Molar TONO yields from
287 1.15±0.09% (pH 6) to 4.93±1.42% (pH 8) were obtained for CTMA, while BZK showed molar
288 conversions of between 0.68±0.24% and 2.12±0.40%, depending on the pH. Among the studied
289 micropollutants, MET showed the lowest TONO yields (≤0.21±0.03%), being at least one order of
290 magnitude lower than for the other compounds, for a given pH value.

291 These results demonstrate the relatively low NDMA formation yields of the studied micropollutants,
292 consistent with the results recently reported by Woods-Chabane et al. (2017) for tertiary and
293 quaternary amines in chloraminated wastewater samples. However, despite the low NDMA formation
294 yield, the studied precursors can produce significant TONO concentrations. The composition of the
295 formed TONO is unknown and is further discussed in section 3.3. Even though the overall mutagenicity
296 potential of the TONO pool cannot be easily evaluated, the molar TONO yields suggest that the
297 corresponding precursors should be taken into consideration during chloramination of wastewaters
298 and drinking waters. Being associated to consumer products, the studied precursors can easily enter
299 the aquatic system by wastewater discharge and get into the drinking water by this way. For example,
300 BZK homologues have been detected in wastewater effluents, river waters and groundwaters at
301 concentrations ranging from 10 ng L^{-1} to 65 µg L^{-1} (Clara et al., 2007; Ding and Liao, 2001; Estévez et
302 al., 2012; Ferrer and Furlong, 2001; Martínez-Carballo et al., 2007). Relevant concentrations
303 (≤0.66 µg L^{-1}) of alkyltrimethylammonium chloride compounds, including CTMA, were found in
304 Taiwanese rivers (Ding and Tsai, 2003), while concentrations in the range 10-150 ng L^{-1} were detected
305 in Austrian surface waters (Martínez-Carballo et al., 2007). CHD concentrations of up to 5 ng L^{-1} have
306 been found in a drinking water reservoirs in Turkey (Yavuz et al., 2015), while concentrations
307 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 μg 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 μg 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 (Garcia 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),

335 *N,N*-dimethylbutyl amine (DMBA), *N,N*-dimethylbenzyl amine (DMBzA) and tetramethyl ammonium
336 (TeMA). Molar NDMA and TONO yields obtained in the pH range 6-8 are reported in Table 2. Similar to
337 the selected micropollutants, for these model compounds, only NDMA was found at detectable
338 concentrations from the US-EPA 8270 standard *N*-nitrosamines mix. Quantifiable NDMA and TONO
339 concentrations were found for all the studied model tertiary amines (TMA, DMBA and DMBzA). TMA
340 displayed TONO concentrations at pH 7 slightly higher than the LOQ, resulting in a molar conversion
341 yield of 0.03%. A NDMA concentration higher than the LOQ was determined only at pH 8, with an
342 average molar yield of 0.10%, while TONO concentrations corresponded to a molar yield of 0.29%. The
343 TONO FP for TMA at pH 8 was higher than the NDMA FP, which is probably due to the accuracy of the
344 two analytical methods and related mainly to the different sample pre-treatment procedures**.** In
345 particular, GC-MS/MS analysis could be affected by a significant loss of NDMA due to the sample
346 extraction procedure, which was not necessary for TONO analysis. A formation of non-NDMA
347 *N*-nitrosamines is not possible from TMA. Furthermore, the TONO method applied in this study
348 (Breider and von Gunten, 2017) tends to slightly overestimate NDMA concentrations (115%±5%
349 recovery of NDMA from standard curve of US-EPA 8270 *N*-nitrosamines standard mix), which may be
350 an additional explanation for the observed discrepancy.
351 NDMA was not detected in DMBA samples at pH 6 and 7, while TONO concentrations slightly above the
352 LOQ (corresponding to 0.02% molar yield) were measured at pH 7. NDMA and TONO molar yields of
353 0.05% and 0.17%, respectively, were obtained at pH 8. Similar to TMA, the NDMA FP observed for
354 DMBA at pH 8 was lower than the TONO FP and the difference in NDMA and TONO concentrations can
355 again be mainly attributed to the differences in the analytical methods.
356 Among the model tertiary amines, DMBzA displayed the highest *N*-nitrosamine formation potentials.
357 Molar NDMA yields were, on average, 1.22% at pH 7 and 5.05% at pH 8, while molar TONO yields were
358 0.72% or 3.27% at pH 7 or 8, respectively. The NDMA FPs resulted to be higher than TONO FPs in this
359 case. However, considering the uncertainties associated to the two analytical methods, measured
360 NDMA and TONO concentrations can be considered to be comparable, indicating that NDMA was the
361 most prevalent *N*-nitrosamine present in the analysed samples. The relatively high formation of NDMA
362 from DMBzA can be attributed to the low stability of the benzyl group, which is easily released from

363 the N-atom (Kemper et al., 2010; Selbes et al., 2013; Spahr et al., 2016). In fact, the benzyl group is a
364 weaker base compared to alkyl groups and plays a stronger electron donating effect on the DMA
365 moiety, resulting in higher NDMA formation rates (Shen and Andrews, 2011a).
366 Comparing the molar yields of NDMA and TONO for all the selected tertiary amines (MET, TMA, DMBA
367 and DMBzA), the role of the molecular structures of the precursors, in particular the type of the
368 functional group bound to the DMA moiety, becomes evident. Even though it was not possible to
369 evaluate the TONO composition, experimental results confirmed that the benzyl functional group is
370 much more easily released than the methyl, butyl or biguanide moieties, leading to significantly higher
371 NDMA yields.
372 For TeMA, the only tested quaternary amine model compound, NDMA concentrations higher than the
373 LOD were never detected, while quantifiable TONO FPs were found at all the pHs. TONO
374 concentrations at pH 6 and 7 were slightly higher than LOQ, while quantifiable concentrations were
375 measured at pH 8, corresponding to a 0.90% molar TONO yield. Since NDMA is expected to be the
376 most prevalent *N*-nitrosamine formed from TeMA, the significant difference between NDMA and TONO
377 yields observed at pH 8 could be explained by an underestimation of NDMA concentration due to the
378 sample extraction procedure. Even though the TONO composition observed for TeMA was not
379 characterized, the results obtained for other precursors show a link between the molecular structures
380 of QACs and *N*-nitrosamine formation. The TONO FP for TeMA was much lower than for the QACs BZK
381 and CTMA, with a significant difference in molar yields (difference >60%, considering the average
382 molar yields reported in Table 2 for pH 8). This indicates that the functional groups bound to the N-
383 atom of QACs play an important role for the *N*-nitrosamine conversion efficiency.
384 A previous study showed that formation of specific *N*-nitrosamines (i.e. NDMA and NDBA) from
385 chloramination of QACs is mostly associated to QACs themselves rather than lower order amine
386 impurities (Kemper et al., 2010). However, the negligible contribution of tertiary amine impurities has
387 not been demonstrated for the TONO FP. The selected tertiary amine model compounds in the current
388 study contain the same, or very similar, functional groups as potential impurities in the QACs.
389 Although the aliphatic chain is shorter in DMBA compared to BZK and CTMA, previous studies have
390 shown that the length of the alkyl group next to the nitrogen atom does not significantly affect the

391 *N*-nitrosamine conversion yield (Selbes et al., 2013). Results of FP tests showed that molar
392 *N*-nitrosamine yields of DMBA and TMA were never high enough to explain *N*-nitrosamine formation
393 from QACs by the presence of impurities (maximum estimated contribution to NDMA or TONO FPs of
394 BZK and CTMA <1%). DMBzA, for which the highest NDMA FP was observed, could contribute up to
395 the 45% of NDMA formation potential of BZK, if considering a maximum content of 5% as impurity
396 (BZK purity >95%). In contrast, the maximum contribution of DMBzA impurities to the TONO FP of
397 BZK would be ≤ 8%, indicating that most of the uncharacterized *N*-nitrosamine production is due to
398 BZK itself. Hence, the results from this study indicate that the observed TONO FP from QACs cannot be
399 attributed to tertiary amine impurities and that QACs themselves are the dominant *N*-nitrosamine
400 precursors.
401
402 **3.3 Elucidation of the potential structures of unknown *N*-nitrosamines formed from**
403 **quaternary ammonium compounds**
404 TONO constituents formed from the selected consumer product ingredients were partially
405 characterized only for BZK, for which it was possible to determine the NDMA FP. The ratio between
406 the average observed molar concentrations of NDMA and TONO was 0.26. Even by taking the
407 differences in the two analytical methods into account, the difference between NDMA and TONO
408 concentrations is significant. This indicates that a large fraction of the total *N*-nitrosamine pool
409 consists of unknown compounds. So far, no known *N*-nitrosamine has been identified in the TONO
410 pools formed from CHD, MET and CTMA.
411 Because no compounds from the US-EPA 8270 standard *N*-nitrosamines mix were found, some other
412 *N*-nitrosamines must be responsible for the measured TONO. Possible *N*-nitrosamine structures
413 formed by CHD cannot be easily proposed due to the complex molecular structure of the precursor. In
414 contrast to most of the tertiary or quaternary amines studied in the literature, CHD does not contain a
415 dialkylamino group which can be related to the formation of the corresponding *N*-nitrosamine, such as
416 NDMA, NDEA or NDBA (Kemper et al., 2010; Le Roux et al., 2011; Selbes et al., 2013; Shen and
417 Andrews, 2011a; Spahr et al., 2016). MET contains similar biguanide moieties as CHD (Table 1),
418 however, it showed significantly lower *N*-nitrosamine FP. Comparing the molecular structures of the

419 two precursors, the higher *N*-nitrosamine formation yield of CHD can be attributed to the aromatic
420 moiety, which is bound to the end-membered N-atom of the biguanide group. In comparison, MET
421 contains the same biguanide structure, however, with end membered DMA moiety. The low NDMA FP
422 of MET has been explained by the electron-withdrawing effect of the biguanide group bound to DMA
423 leading to a lower nucleophilicity of the N-atom (Le Roux et al., 2011; Selbes et al., 2013; Shen and
424 Andrews, 2011a). In contrast, the chlorinated aromatic ring in the CHD structure could enhance the
425 nucleophilic attack on the chloramine. Nevertheless, further studies are needed to verify these
426 assumptions.
427 For QACs, the presence of only one N-atom limits the number of possible reactions with NH_2Cl leading
428 to potential *N*-nitrosamine products. It has been demonstrated that *N*-nitrosamine formation from
429 tertiary and quaternary amines involves the release of the functional groups bound to the nitrogen
430 atom in the precursor molecule (Selbes et al., 2013). Various studies have shown that the structure of
431 the leaving groups in precursors containing a DMA moiety strongly influences the formation of NDMA,
432 resulting in a large range of formation yields (Kemper et al., 2010; Le Roux et al., 2011; Selbes et al.,
433 2013; Shen and Andrews, 2011b). However, most of these studies focused on NDMA formation and
434 TONO measurements were rarely performed.
435 In the current study, the structures of other potential *N*-nitrosamines were hypothesized based on all
436 possible leaving groups present in the precursor molecule. The potential *N*-nitrosamines resulting
437 from chloramination of BZK and CTMA are shown in Table 3. For instance, the benzyl group and the
438 aliphatic chain (C_nH_{2n+1}) bound to the N-atom in BZK are supposed to act as leaving groups during
439 NDMA formation. Similarly, the release of one methyl group and the 16-C aliphatic chain ($C_{16}H_{33}$) may
440 lead to the formation of NDMA from CTMA. The other *N*-nitrosamine structures were proposed by
441 considering all the other potential leaving groups, including the methyl group, the benzyl group and
442 the long-chain alkyl group for BZK, the methyl groups and the 16-C aliphatic chain for CTMA.
443 To test the formation of the proposed *N*-nitrosamines, the samples obtained by the TONO FP tests at
444 pH 8 were analysed by high resolution LC-qTOF. The high accuracy and resolution of the obtained
445 mass spectra allow proposing a compound based on its exact mass, in absence of analytical reference
446 standards. The procedure described in section 2.3 was applied to screen the list of potential *N*-

447 nitrosamines from the chromatograms, by extracting their exact masses (*m/z*) (calculated by the
448 ChemCalc software and reported in Table 3, including the protonated molecules). The procedure was
449 also applied to detect potential denitrosation products of the investigated compounds.
450 Among the hypothesized *N*-nitrosamines, a positive match was obtained for BZK. The full
451 chromatogram of the BZK sample (treated with 10-fold molar excess of monochloramine at pH 8) is
452 reported in Figure S3, SI. The extraction of the exact mass of $C_{13}H_{28}N_2O$ ($[M+H]^+$=229.2279) allowed to
453 obtain a distinct peak (intensity of $8.56 \cdot 10^5$ arbitrary units) at a retention time of 7.34 min (Figure
454 2 a). The relative mass spectrum (Figure 2 b) showed a peak at *m/z*=229.2271, corresponding to a
455 relative abundance of 93% and matching with high accuracy (error<0.1%) with two possible
456 molecular formulas: (i) the hypothesized *N*-nitrosamine ($C_{13}H_{28}N_2O$, error=3.876 ppm, Table 3) and
457 (ii) $C_{11}H_{27}N_5$ (error=1.981 ppm). The formation of the latter compound is highly improbable
458 considering the investigated reaction system. The identified mass corresponded with a high level of
459 confidence to *N*-nitroso-*N*-methyldodecylamine (Figure 2 a), a potential product of the reaction of
460 chloramine with benzyldimethyldodecylamine, which is the most abundant constituent of the
461 benzalkonium chloride mixture (about 70% abundance, according to the supplier (Sigma-Aldrich)).
462 The detection of *N*-nitroso-*N*-methyldodecylamine was not unexpected and its formation at significant
463 yield is consistent with previous results reported in the literature (Kemper et al., 2010; Selbes et al.,
464 2013; Spahr et al., 2016). Because the benzyl group is an electron-donating group, which increases the
465 electron density on the N-atom, a nucleophilic attack on the chloramine is favoured. In addition,
466 benzyl-type functional groups are known to be better leaving groups than alkyl groups, both in tertiary
467 and quaternary amine precursors (Kemper et al., 2010; Selbes et al., 2013). Hence, during
468 chloramination of BZK homologues, *N*-nitroso-*N*-metylalkylamines are expected to have higher yields
469 than benzyl-containing *N*-nitrosamines. Consistently, it was found previously that the molar yield of
470 *N*-nitrosomethylbenzylamine from benzyldimethyltetradecylamine (the C-14 constituent of BZK) was
471 only 0.016%, about 16-times lower than the molar NDMA yield (Kemper et al., 2010).
472 High resolution LC-qTOF analysis of a CTMA sample did not lead to an identification of the
473 hypothesized compound ($C_{17}H_{36}N_2O$) with enough confidence (results are reported in Figures S3 and
474 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 mg kg^{-1} 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 mg kg^{-1} d^{-1} of NDMA to 0.57 mg kg^{-1} 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

503 realistic systems, leading to the formation of extremely high *N*-nitrosamine concentrations. To validate
504 the molar TONO yield values obtained in ES1, FP tests were also conducted in ES2, with experimental
505 conditions closer to real water matrices (pH 8, 0.5 μM precursor and 100 μM monochloramine,
506 corresponding to 5.1 mg L^{-1} as NH_2Cl). The relatively long reaction time in FP tests (7 days) was mainly
507 selected to maximize *N*-nitrosamine formation and can be considered as an upper limit of residence
508 times in drinking water distribution systems. In contrast, it is longer than typical contact times for
509 wastewater disinfection. Due to the lower *N*-nitrosamine concentrations produced under ES2, TONO
510 concentrations were measured after sample pre-concentration by solid phase extraction (SPE).
511 As discussed above, the composition of the generated TONO compound pool is different for each
512 precursor. Since the physico-chemical properties of the formed *N*-nitrosamines can strongly affect the
513 efficiency of SPE (Dai and Mitch, 2013), the recovery of TONO concentrations by SPE was determined
514 for the specific *N*-nitrosamine pools formed by each precursor, as reported in section 2.2. The TONO
515 recovery efficiencies (values reported in Table S2, SI) resulted to be quite low for all the precursors,
516 with values of about 10%. Applying the obtained recovery efficiency values to the TONO
517 concentrations measured in the FP tests (ES2), the molar TONO yields resulted, on average as
518 6.54±0.39%, 4.05±0.38%, 4.05±0.02% and 0.77±0.32% for CHD, BZK, CTMA and MET, respectively.
519 Despite the extremely different experimental conditions and the uncertainty associated to SPE, the
520 molar TONO yields obtained under ES2 were comparable with those obtained in ES1 at pH 8 (Table 2).
521 CHD and MET displayed the highest and the lowest molar TONO yields, respectively, while
522 intermediate values were obtained for QACs. Even though the molar TONO yields are slightly different,
523 they are in the same order of magnitude for ES1 and ES2. This indicates that results obtained in ES1
524 can be considered representative for more realistic conditions.
525 When dealing with TONO measurements in real water matrices, the recovery efficiency of SPE plays a
526 key role and it can strongly vary with the TONO pool composition (Dai et al., 2015; Zeng and Mitch,
527 2015). In this study, the SPE extraction efficiency was also evaluated for a 0.16 μM US-EPA 8270
528 standard *N*-nitrosamine mix solution, both in terms of TONO and specific *N*-nitrosamine constituents.
529 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*-methyldodecylamine) 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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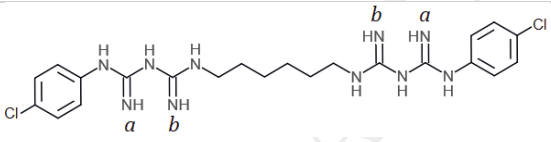
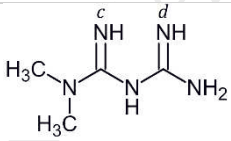
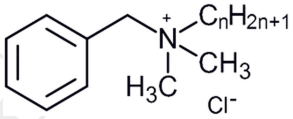
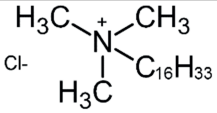
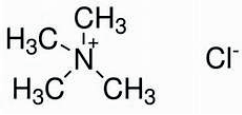
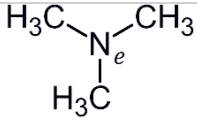
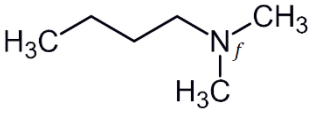
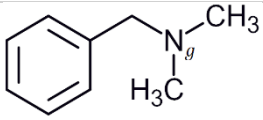
Table 1 Selected micropollutants and model compounds: molecular structure, molecular weight (MW) and experimentally determined pK_a values (references given in the footnotes). For CHD and MET, the pK_a values are referred to the equilibrium $C{=}NH_2^+ \leftrightarrows C{=}NH + H^+$.

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)	 (n=8, 10, 12, 14, 16, 18)	283.4-423.4 (average 348.4)	-
Cetyl trimethyl ammonium chloride (CTMA)		319.4	-
Model compounds			
Tetramethyl ammonium chloride (TeMA)		109.6	-
Trimethyl amine (TMA)		59.1	9.80 [e]
N,N-dimethylbutyl amine (DMBA)		101.2	10.19 [f]
N,N-dimethylbenzyl amine (DMBzA)		135.2	8.91 [g]

[a, b] (Agarwal et al., 2012)

773 [c, d] (Devi, 2013)
774 [e, f, g] ChemlDplus-Toxnet database (https://chem.nlm.nih.gov/chemidplus/).
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778 **Table 2** Molar yields of NDMA and TONO observed in formation potential (FP) experiments under
779 experimental setup ES1 (reaction time: 7 days; initial precursor concentration: 0.1 mM for CHD,
780 0.5 mM for the other compounds; initial NH_2Cl molar excess: 10-fold for BZK, 200-fold for the other
781 compounds) and ES2 (reaction time: 7 days; initial precursor concentration: 0.5 μM; initial NH_2Cl
782 molar excess: 200-fold). Reported molar yields are the average values of multiple measurement
783 repetitions (two for NDMA and three for TONO) on two experimental replicates.

Precursor	**pH**	**Molar yield (%)**		
		NDMA [a] average (range)	**TONO [b]** average ± st.dev.	
		ES1	**ES1**	**ES2**
Micropollutants				
Chlorhexidine hydrochloride (CHD)	6	<0.09	2.64 ± 1.45	-
	7	<0.09	4.93 ± 1.33	-
	8	<0.09	11.92 ± 0.83	6.54 ± 0.39
Metformin dihydrochloride (MET)	6	<0.09	0.04 ± 0.003	-
	7	<0.09	0.09 ± 0.01	-
	8	<0.09	0.21 ± 0.03	0.77 ± 0.32
Benzalkonium chloride (BZK)	6	<0.09	0.68 ± 0.24	-
	7	<0.09	1.31 ± 0.21	-
	8	0.56 (0.55-0.56)	2.12 ± 0.40	4.05 ± 0.38
Cetyl trimethyl ammonium chloride (CTMA)	6	<0.09	1.15 ± 0.09	-
	7	<0.09	4.77 ± 1.00	-
	8	<0.09	4.93 ± 1.43	4.05 ± 0.01
Model compounds				
Tetramethyl ammonium chloride (TeMA)	6	<0.09	0.04 ± 0.002	-
	7	<0.09	0.07 ± 0.004	-
	8	<0.09	0.90 ± 0.08	-
Trimethyl amine (TMA)	6	<0.09	<1.0	-
	7	<0.09	0.03 ± 0.005	-
	8	0.10 [c]	0.29 ± 0.007	-
N,N-dimethylbutyl amine (DMBA)	6	<0.09	<1.0	-
	7	<0.09	0.02 ± 0.004	-
	8	0.05 [c]	0.17 ± 0.03	-
N,N-dimethylbenzyl amine (DMBzA)	6	<0.09	<1.0	-

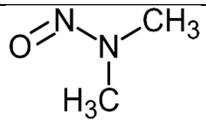
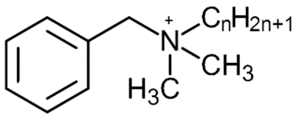
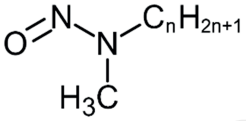
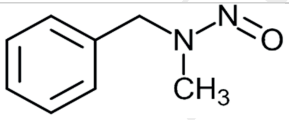
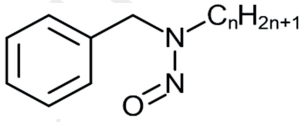
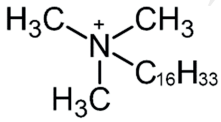
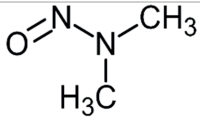
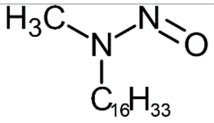
	7	1.22 (1.08-1.36)	0.72 ± 0.03	-
	8	5.05 (3.92-6.18)	3.27 ± 0.34	-

[a] LOQ for NDMA: 0.09±0.0001 µM.

[b] LOQ for TONO: 1.0±0.45 µM.

[c] NDMA concentration >LOQ only in one sample.

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 *N*-nitrosamines			
	Structure	Formula	Exact mass (*m/z*)	
			$[M]^+$	$[M+H]^+$
	 (NDMA)	$C_2H_6N_2O$	74.0480	75.0558
BZK  n=8, 10, 12, 14, 16, 18	 n=8, 10, 12, 14, 16, 18	$C_{n+1}H_{2n+4}N_2O$ 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
		$C_8H_{10}N_2O$	150.0793	151.0871
		$C_{n+7}H_{2n+8}N_2O$ 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 	 (NDMA)	$C_2H_6N_2O$	74.0480	75.0558
		$C_{17}H_{36}N_2O$	284.2828	285.2906

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800 **Figure captions**

801 **Figure 1** Kinetics of TONO formation at pH 7 for (a) CHD, (b) BZK, (c) CTMA and (d) MET as a function
802 of the initial molar NH_2Cl excess: 10- (△), 20- (◇), 40- (×), 100- (□) and 200-fold (○) NH_2Cl molar
803 excess. The initial precursor concentration was set at 0.1 mM for CHD and 0.5 mM for the other
804 compounds. Data represent the average ± standard deviation of three measurement repetitions on two
805 experimental replicates.

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

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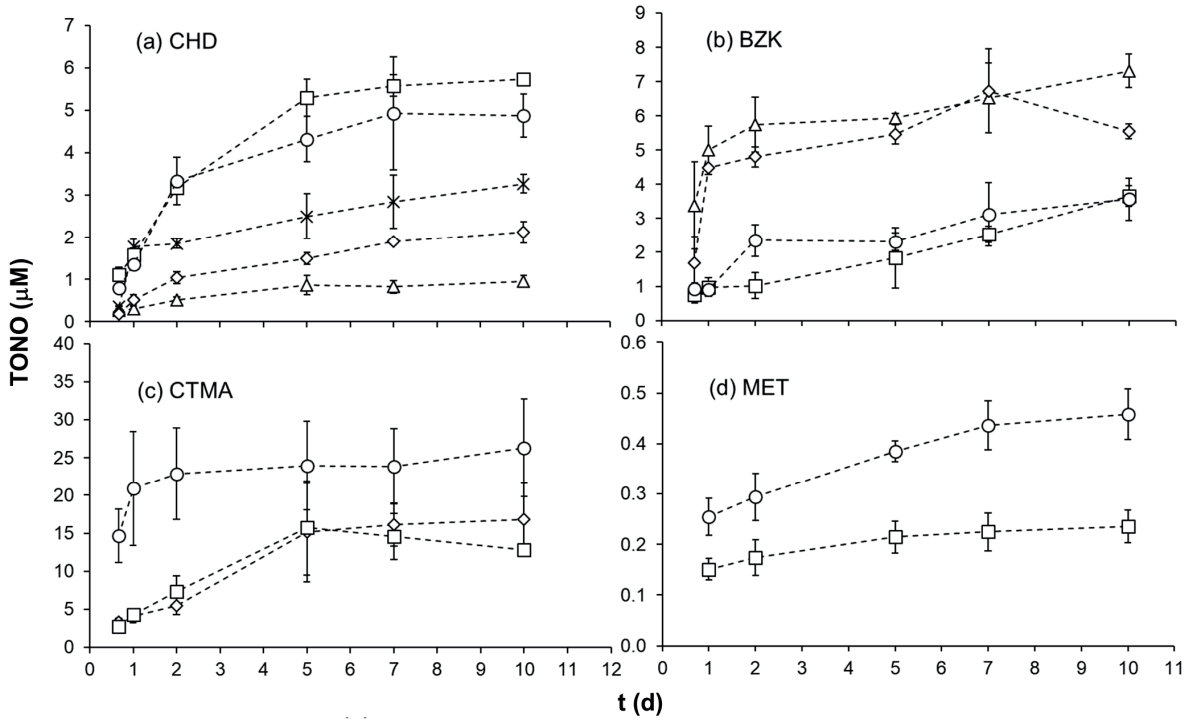

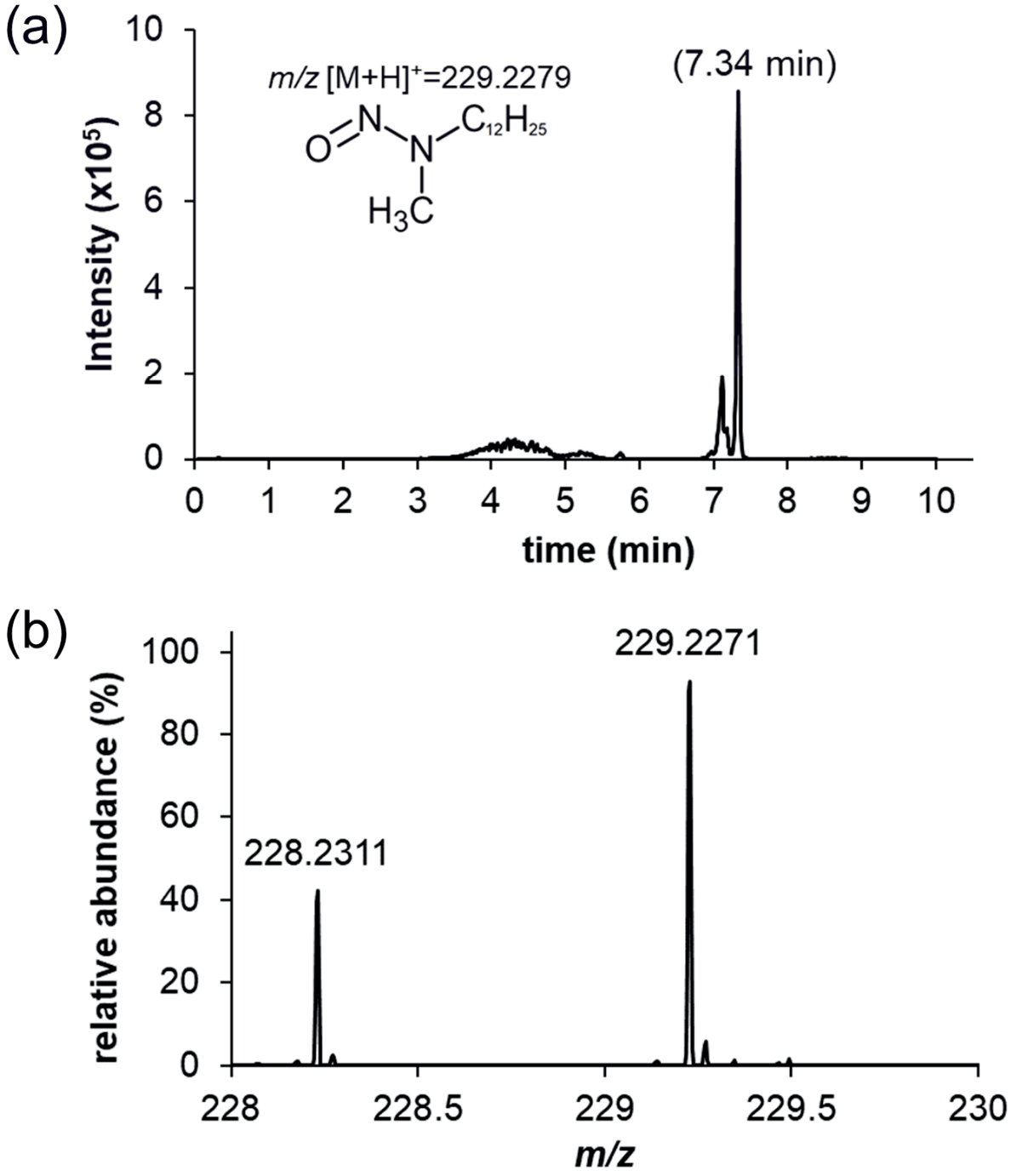
(a)
m/z [M+H]+=229.2279
(7.34 min)
Intensity (x10^5)
time (min)
(b)
229.2271
228.2311
relative abundance (%)
m/z

- Total *N*-nitrosamine (TONO) formation yield of various micropollutants was determined
- TONO pools were mostly uncharacterized, only NDMA could be quantified
- A LC-qTOF screening procedure was used to characterize the TONO pool
- *N*-nitrosamine formation yield depends on the moieties in precursor molecule