

Environmental risk assessment for quaternary ammonium compounds: a case study from Austria

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Abstract Quaternary ammonium compounds (QAC) are widely used as disinfectants, biocides, and detergents, among a variety of other applications. The cationic surface-activity of QAC determines their potential to act as a biocide on both target and non-target organisms. This study aims to provide a broad-based environmental risk characterization and evaluation for selected QAC with particular focus on the situation in Austrian rivers. A modular study design was employed involving environmental exposure characterization, QAC fate and effect analysis in wastewater, ecotoxicological effect characterization, and environmental risk evaluation. A wide array of Austrian surface waters and wastewater effluents were screened for the selected key compounds, benzalkonium chlorides and dialkyldimethylammonium chlorides with different C-chain lengths. Ecotoxicological effect characterization was based on both microbioassays for a set of representative aquatic organisms and a literature review. For risk evaluation, the PEC/PNEC ratio was above 1 for some rivers. Notably, small rivers with a high particulate matter were contaminated with QAC in the case of high water incidents. Hence, for the Austrian sites studied, a QAC-derived risk to sensitive aquatic non-target organisms could not be excluded.

Keywords Quaternary ammonium compounds; risk assessment; water pollution; wastewater treatment

Introduction

Quaternary ammonium compounds (QAC) form an economically important class of industrial chemicals that are widely distributed among a diverse array of products and users from the industrial to the household sectors. Because of their strong cationic surface activity, QAC are used primarily as disinfectants, biocides, and detergents, but also as anti-electrostatics, and as phase transfer catalysts among others. In Germany, 1 520 t dihydrogenated tallow dimethyl ammonium chloride (DHTDMAC consisting of 60–70% C18 chains) were produced in 1994 (European Union, 2002). Madsen *et al.* (2001) estimated the annual consumption of cationic surfactants in Europe (1998) in industrial and institutional products as 17 000 t and in household products as 98 000 t. QAC are typically released into wastewater treatment plants (WWTPs) from where they may be discharged. In the aquatic environment, QAC are characterized by a high potential to adsorb to organic or inorganic surfaces such as suspended solids, sediments, and organisms. Hence, the strong beneficial biocide properties of QAC may pose a threat to non-target organisms in the activated sludge of WWTPs, as well as those dwelling in natural surface waters. For reviews, see e.g. Boethling and Lynch (1992), García *et al.* (2001), and Petrovic and Barceló (2004).

The aim of this study was to form a basis for environmental risk characterization and evaluation for selected QAC compounds with particular focus on the situation in Austrian rivers in relation to WWTP effluents. Effluents from WWTPs differing in size and plant layout were analysed for QAC concentrations. Ecotoxicological effect characterization was based on experimental determination of effective concentrations employing standardized biotesting procedures for a set of representative aquatic organisms (algae, ciliate protozoans, rotifers, and daphnids) and a literature review.

The results for this study were obtained in the course of a large-scale Austrian joint research project on a risk assessment for QAC. This project employed a modular study design that involved (1) environmental exposure characterization, (2) QAC fate and effect analysis in the wastewater cycle, (3) ecotoxicological effect characterization, and (4) risk evaluation. New methods developed for chemical analysis and detailed results on fate and effects of the QAC in the compartments studied were submitted for publication (e.g. Martínez *et al.* submitted). With respect to their physical and chemical characteristics, the QAC form a highly diverse group of chemicals. For this study, benzalkonium chlorides (BAC) and dialkyldimethylammonium chlorides (DDAC) were selected as key compounds because of their product and application profiles, as well as their ecotoxicological properties. The selection was based on information provided by the manufacturers in questionnaires and retrieved from the literature.

Methods

Chemical analysis

Water samples were characterized for conventional wastewater parameters applying standardized methods. Analyses of BAC-C12 to BAC-C18 and DDAC-C10 to DDAC-C18 were performed with a procedure similar to an ion-pair extraction according to Radke *et al.* (1999). Water samples (500 mL) were extracted with chloroform after acidification with HCl to a pH < 1. After evaporation and resolution with chloroform, the extract was washed with deionized water, evaporated, and the residue dissolved in methanol. For separation, an LC system, Agilent 1100 HPLC, was utilized equipped with an analytical column (Luna C18 Phenomenex) and for detection, a Quattro Ultima triple quadrupole mass spectrometer equipped with an electrospray interface from Micromass (Manchester, UK) was in use.

For the quantification of QAC in the analytical processes, single compound standards (Aldrich or Fluka) of benzalkonium chlorides (BAC-C12, BAC-C14, BAC-C16, BAC-C18) and dialkyldimethylammonium chlorides (DDAC-C10, DDAC-C12, DDAC-C14, DDAC-C16, DDAC-C18) were used. For chromatographic separation of BAC-C12 to BAC-C18 and DDAC-C10 to DDAC-C18, a LC/MS–MS system was used with the limits of detection (LOD) and limits of quantification (LOQ) given in Table 1. Chemical analyses were conducted at the Umweltbundesamt Wien (Austria).

Sampling

Wastewater treatment plants (WWTPs). Effluent samples were taken from five municipal WWTPs (WWTP 1–5) that were selected according to their location on receiving waters

Table 1 Limits of detection (LOD) and limits of quantification (LOQ) for selected QAC

	DDAC-C10	DDAC-C12	DDAC-C14	DDAC-C16	DDAC-C18
LOD (µg/L)	0.006	0.006	0.006	0.006	0.011
LOQ(µg/L)	0.012	0.011	0.011	0.011	0.022
	BAC-C12	BAC-C14	BAC-C16	BAC-C18	
LOD (µg/L)	0.006	0.006	0.006	0.006	
LOQ (µg/L)	0.012	0.012	0.012	0.011	

of different size, relevant single source emitters, and plant layout. A characterization of the WWTPs sampled is given in Table 2 including effluent concentrations for relevant nutrients.

Flow-proportional, 24 h-composite samples were taken from the effluent of the respective WWTPs. The samples were stabilized with sodium acid and chloroform and stored in the fridge. Samples were collected in two sampling periods (June and September 2004) for most of the WWTPs.

The sampling was performed by the Vienna University of Technology and the University of Natural Resources and Applied Life Sciences, Vienna.

Surface water sampling. Grab samples of surface water sites (Table 3) were selected to correspond effluents of WWTPs approximately 1 km downstream of the effluent. The samples were stabilized with sodium acid and chloroform and stored in the fridge. Surface water samples were collected in two sampling periods (June and September 2004). In June 2004, surface waters showed higher than average discharge and high turbidity after rainfall during two days before sampling. The samples in September 2004 were taken after two weeks without rainfall and below average discharge conditions. The sampling was performed by the Vienna University of Technology.

Biotest

To assess the ecotoxicity of QAC to freshwater non-target organisms, we employed the single-species, shorter term, static exposure Toxkit-Biotest technology (MicroBioTests Inc., Nazareth, Belgium). Effects on growth and reproduction were tested for green algae (*Pseudokirchneriella subcapitata*; syn. *Selenastrum capricornutum*, *Raphidocoelis subcapitata* 72 h EC₅₀), ciliate protozoans (*Tetrahymena thermophila*; 24 h EC₅₀), and rotifers (*Brachionus calyciflorus*; 48 h EC₅₀). Daphnids were tested for acute immobilization (*Daphnia magna*; 48 h IC₅₀). Test protocols followed established guidelines (e.g. OECD). Biotests were conducted at the University of Veterinary Medicine of Vienna.

Test compounds were a BAC mixture (ca. 60% BAC-C12, 40% BAC-C14, <1% BAC-C16; purity ≥ 95%; CAS Nr 8001-54-5; Sigma - Aldrich) and dimethyl-ditetradecylammonium bromide (DDAB-C-14; purity ≥ 97%; CAS Nr. 68105-02-2; Fluka). Effect concentrations were calculated for both nominal and actual concentrations (mean recovery was 78.3% for BAC and 34.5% for DDAC).

Results and discussion

The concentrations of BAC and DDAC obtained by HPLC analyses in surface waters are given in Tables 4 and 5. BAC with a C-chain length of C12 and C14 and DDAC with a C-chain length of C10 and C18 were most frequently detected. This observation parallels the pattern of use of QAC, as also detected in single source pollution samples (data not shown), as well as in technical mixtures commonly applied in industrial uses.

In surface water samples, concentration levels of the single substances were determined below 1 µg/L with one exception (L1 - Liesing above WWTP 1.9 µg/L BAC-C12). The concentrations of BAC are basically higher than those of DDAC. The medians of the single substances in the surface water samples are generally lower than 0.1 µg/L. Osburn (1982) and Topping and Waters (1982) investigated cationic detergents in surface water applying the disulfine blue method and found concentrations in the range of 4–42 µg/L.

DDAC and BAC concentrations under high flow conditions in the rivers generally were significantly higher compared to low flow conditions for all sites, with the exception of D2. D2 is situated on the right bank of the river Danube within the effluent plume of the (at that time) high loaded Vienna Main Sewage Treatment Plant. During low flow

Table 2 Description of WWTPs sampled

WWTP	Size (PE)	Characteristics		Effluent Jun.04 (mg/L)				Effluent Sept. 04 (mg/L)			
				COD	NH ₄ -N	NO ₃ -N	PT	COD	NH ₄ -N	NO ₃ -N	PT
WWTP 1	130 000	PS, AB, AD	C, P, N, DN	20	0.11	0.9	0.23	34	0.2	1	1.9
WWTP 2	2 500 000	PS, AB	C, P	120	23	1.7	1.3	77	35.5	2.4	1.7
WWTP 3	95 000	PS, AB, AD	C, P, N, DN	26	1.1	3.6	0.4				
WWTP 4	300 000	PS, AB	C, P, N	29.5	4	5	0.3				
WWTP 5	230 000	PS, AB, AD	C, P, N, DN	54	1.1	3.8	1	42	2.1	4.9	1.7

PS = primary sedimentation; AB = aeration basin; AD = anaerobic digestion; C = carbon removal; P = phosphorus precipitation; N = nitrification; DN = denitrification; PE = population equivalents

Table 3 Abbreviation for surface water samples used in the results and discussion section

Site abbreviation	Site description
L1	Liesing (upstream WWTP)
L2	Liesing (downstream WWTP)
Y1	Ybbs–Amstetten (upstream WWTP)
Y2	Ybbs–Gleis (downstream WWTP)
Y3	Ybbs–Neumarkt (mouth into Danube)
S1	Schwechat–Helenental (pristine)
S2	Schwechat–Maria Lanzendorf
S3	Schwechat–Albern (mouth into Danube)
D1	Danube–Kritzendorf (upstream Vienna)
D2	Danube–Albern Hafen (downstream Vienna)
D3	Danube–Haslau (15 km downstream D2)

conditions in September, there is less dilution by river water and subsequently higher influence of the effluent of that WWTP on the concentration of the surface water, hence repelling the influence of the WWTP during higher flow in the Danube.

As there was heavy rainfall during two days previous to sampling, the higher concentration of QACs during high flow conditions may be attributed to stormwater overflow from raw sewage into the receiving waters.

At low flow conditions, most of the concentrations could be observed to be below LOD for sampling sites upstream to the WWTPs. Only L1 showed higher concentrations for DDACs upstream to the WWTP under those conditions. Tables 4 and 5 indicate that L1 is the site with the highest concentrations for all QACs, not only during high flow conditions. The Liesing river (L1 and L2) is a small creek within the southern part of Vienna receiving stormwater overflow and misconnections from sewerages.

Beside Liesing, the river Schwechat showed comparable high concentrations. River Schwechat drains a densely populated area south of Vienna and receives the discharges of WWTPs with a total of approximately 600 000 PE with only low dilution. This results in the high concentrations analysed for S2 and S3.

Surface water sites located downstream of WWTPs showed slightly higher concentrations under both high, as well as low flow conditions in the surface waters. The influence of WWTPs can be seen during the sampling in September, where in most cases, upstream concentrations of QACs <LOQ are observed, while downstream of WWTPs concentrations >LOQ were analysed.

Results for WWTP effluents are shown in Tables 6 and 7, respectively.

Table 4 Results for DDAC measurements in surface waters–June/September 2004 [$\mu\text{g/L}$]

	DDAC-C10 Jun.04/Sep.04	DDAC-C12 Jun.04/Sep.04	DDAC-C14 Jun.04/Sep.04	DDAC-C16 Jun.04/Sep.04	DDAC-C18 Jun.04/Sep.04
L1	0.15/0.12	0.022/<LOD	<LOD/<LOD	0.05/0.021	0.083/0.054
L2	0.081/<LOD	0.013/<LOD	<LOD/<LOD	0.044/<LOQ	0.077/0.034
Y1	0.033/<LOD	0.011/<LOD	<LOD/<LOD	<LOQ/<LOD	0.022/<LOD
Y2	0.046/0.13	0.019/<LOD	<LOD/<LOD	0.018/<LOD	0.022/<LOD
Y3	0.024/<LOQ	<LOQ/<LOD	<LOQ/<LOD	<LOQ/<LOD	0.022/<LOD
S1	0.031/<LOD	<LOQ/<LOD	<LOD/<LOD	<LOD/<LOD	<LOQ/<LOD
S2	0.069/<LOQ	0.015/<LOD	<LOQ/<LOD	0.05/0.012	0.19/0.031
S3	0.012/<LOQ	<LOD/<LOD	<LOQ/<LOD	0.012/<LOQ	0.033/0.028
D1	0.022/<LOD	<LOD/<LOD	<LOD/<LOD	<LOQ/<LOD	<LOQ/<LOD
D2	0.015/0.69	<LOD/<LOD	<LOD/<LOD	<LOD/<LOQ	<LOD/<LOQ
D3	0.017/<LOQ	<LOD/<LOD	<LOQ/<LOD	<LOD/<LOQ	<LOQ/<LOD
LOD	0.006	0.006	0.006	0.006	0.011
LOQ	0.012	0.011	0.011	0.011	0.022

Table 5 Results for BAC measurements in surface waters–June/September 2004 [$\mu\text{g/L}$]

	BAC-C12	BAC-C14	BAC-C16	BAC-C18
	Jun.04/Sep.04	Jun.04/Sep.04	Jun.04/Sep.04	Jun.04/Sep.04
L1	1.9/0.045	0.51/0.05	0.11/0.44	0.094/0.018
L2	0.097/0.067	0.074/0.034	0.1/<LOQ	0.086/<LOQ
Y1	0.027/<LOQ	0.012/<LOQ	0.012/<LOD	0.014/<LOD
Y2	0.025/0.025	0.024/0.031	0.016/<LOQ	0.019/<LOD
Y3	0.015/<LOQ	0.016/0.014	0.012/<LOD	0.013/<LOD
S1	0.048/<LOD	0.021/<LOD	0.012/<LOD	0.014/<LOD
S2	0.29/0.026	0.1/0.026	0.061/<LOD	0.066/<LOQ
S3	0.11/0.06	0.047/0.031	0.03/<LOQ	0.037/<LOQ
D1	0.036/<LOD	<LOQ/<LOD	<LOQ/<LOD	<LOD/<LOD
D2	0.067/0.14	0.026/0.076	<LOQ/<LOQ	<LOQ/0.018
D3	0.052/0.025	0.018/0.014	<LOQ/<LOQ	<LOQ/0.011
LOD	0.006	0.006	0.006	0.006
LOQ	0.012	0.012	0.012	0.012

In the effluent of the treatment plants, highest concentrations were observed for BAC-C12 and DDAC-C10 with $4.1 \mu\text{g/L}$ and $0.85 \mu\text{g/L}$, respectively. Not taking into consideration results from WWTP 2, a significant correlation ($p < 0.05$) could be found between the effluent data from June (heavy rains) and September (dry weather), the effluent data from September being slightly higher. The situation at WWTP 2 is different to the other WWTPs. In contrast to the other WWTPs, the hydraulic capacity of the secondary clarifiers during rain conditions is exceeded, resulting in the discharge of high amounts of TSS into the receiving water. TSS in the effluent of WWTP 2 were 71.3 mg/L during rainy conditions in June compared to 18.7 mg/L in September. Mean TSS concentration in the effluents of other plants were 15.3 mg/L in June and 3.25 mg/L in September. As total unfiltered effluent samples were analysed and the QACs being highly adsorptive, the higher QAC effluent concentrations during rainy weather can be attributed to the loss of TSS in the WWTP 2.

Table 6 Results for DDAC measurements in WWTP effluents–June/September 2004 [$\mu\text{g/L}$]

	DDAC-C10	DDAC-C12	DDAC-C14	DDAC-C16	DDAC-C18
	Jun.04/Sep.04	Jun.04/Sep.04	Jun.04/Sep.04	Jun.04/Sep.04	Jun.04/Sep.04
WWTP 1	0.024/0.096	<LOQ/0.078	<LOD/0.07	<LOQ/0.083	0.026/0.096
WWTP 2	0.85/0.27	0.016/0.08	<LOD/0.053	0.06/0.08	0.21/0.12
WWTP 3	0.027	0.012	<LOQ	0.017	0.054
WWTP 4	0.025	<LOD	<LOD	<LOQ	0.028
WWTP 5	0.078/0.47	<LOQ/0.16	0.26/0.16	0.024/0.17	<LOQ/0.12
LOD	0.006	0.006	0.006	0.006	0.011
LOQ	0.012	0.011	0.011	0.011	0.022

Table 7 Results for BAC measurements in WWTP effluents–June/September 2004 [$\mu\text{g/L}$]

	BAC-C12	BAC-C14	BAC-C16	BAC-C18
	Jun.04/Sep.04	Jun.04/Sep.04	Jun.04/Sep.04	Jun.04/Sep.04
WWTP 1	0.15/0.17	0.052/0.15	<LOQ/0.11	<LOD/0.09
WWTP 2	0.89/0.4	0.61/0.27	0.16/0.13	0.085/0.095
WWTP 3	4.1	1.6	0.69	0.048
WWTP 4	0.081	0.026	0.038	<LOQ
WWTP 5	0.14/1.1	0.098/0.86	0.026/0.31	<LOQ/0.23
LOD	0.006	0.006	0.006	0.006
LOQ	0.012	0.012	0.012	0.011

Table 8 Results from acute immobilization tests (daphnids) and growth inhibition (reproduction) tests with algae, protozoans, and rotifers. Effective concentrations for the QAC test compounds BAC and DDAC are derived from the standardized single species biotests in this study (median effective concentration; mg/L nominal effective concentrations and lowest comparable concentrations reported in the literature)

Species and endpoints studied		BAC $\mu\text{g/L}$	DDAC $\mu\text{g/L}$	Reference
Algae				
<i>Pseudokirchneriella subcapitata</i>	72 h EC50	52	61	This study
<i>Chlorella pyrenoidosa</i>	96 h EC10	670 ^a	6,000 ^b	Utsunomiya <i>et al.</i> (1997)
Protozoans				
<i>Tetrahymena thermophila</i>	24 h EC50	3,755	12,839	This study
<i>Colpoda aspera</i>	24 h EC50	4,310 ^c	2,890 ^c	Kakiichi <i>et al.</i> (1991)
Rotifers				
<i>Brachionus calyciflorus</i>	48 h EC50	160	73	This study
Daphnids				
<i>Daphnia magna</i>	48 h IC50	52	66	This study
<i>Daphnia magna</i>	48 h IC50	400 ^b		Hayes <i>et al.</i> (1993)
<i>Daphnia magna</i>	48 h IC50		30 ^c	Farrell and Kennedy (1999)

^aC12–14; 97.5%

^bC16–18; 97.5%

^cC-n; 10%

^dC-n; 80–82% purity

In the ecotoxicological effect characterization, both compounds tested showed the same pattern of species sensitivities ranking from the most sensitive algae (*P. subcapitata*, 72 h EBC50: 41 $\mu\text{g BAC/L}$, 21 $\mu\text{g DDAC/L}$) and daphnids (*D. magna*, 48 h IC₅₀: 41 $\mu\text{g BAC/L}$, 23 $\mu\text{g DDAC/L}$), the rotifers (*B. calyciflorus*, 48 h EC₅₀: 125 $\mu\text{g BAC/L}$, 25 $\mu\text{g DDAC/L}$) to the least sensitive protozoans (*T. thermophila*, 24 h EC₅₀: 2 941 $\mu\text{g BAC/L}$, 4 427 $\mu\text{g DDAC/L}$; all values are based on actual concentrations). For nominal effect concentrations, own results were compared with those reported in the literature (Table 8). However, a high percentage of the data published so far were not appropriate for comparison because of highly divergent study designs (e.g. test organisms and duration of exposure) or data deficiency (e.g. composition and purity of the test compounds, and lack of information on actual concentrations). Overall, results derived from own experiments were consistent with those reported in the literature, though located at the lower range of variation reported so far.

It needs to be stressed that the lowest actual ecotoxicological effective concentrations determined (41 $\mu\text{g BAC/L}$) exceeded the highest concentrations measured in surface waters (1.9 $\mu\text{g BAC/L}$) and effluents (1.6 $\mu\text{g BAC/L}$) by only one order of magnitude. Considering the proximity of exposure and effect concentrations in the aquatic phase, the scarcity of reliable and comparable data for the QAC studied, and their high potential to adsorb to particulate matter, which is highly relevant for microphagous filter feeding and sediment dwelling organisms, risk from QAC cannot be excluded for aquatic non-target organisms.

Conclusions

In Austria, surface waters and sewage treatment plant effluents were analysed for QAC for the first time. Detection limits and quantification limits ranged as low as 0.006 $\mu\text{g/L}$ and 0.01 $\mu\text{g/L}$, respectively. Water samples with high loads of suspended solids showed QAC concentrations above 1 $\mu\text{g/L}$ (BAC-C12, BAC-C14, DDAC-C10, DDAC-C18) but median values below 100 ng/L.

In essence, the database available for QAC environmental risk assessment is still fragmentary, especially for longer term exposure effects of QAC on reproduction, and development and growth of aquatic organisms, as well as for possible interactions. In addition, our review revealed considerable deficiencies in the reproducibility of the results reported in the literature. Therefore, high assessment factors were necessary for calculation of PNEC values.

For risk evaluation, the PEC/PNEC ratio was above 1 for some river waters (L1), which indicated a potential risk for the aquatic environment. Notably, small rivers were contaminated in the case of high water incidents. The sediment concentrations essentially paralleled those in corresponding surface waters. Hence, for the Austrian sites studied, QAC-derived threat to sensitive aquatic non-target organisms could not be excluded.

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