

Methodological approach towards the environmental significance of uncharacterized substances — quaternary ammonium compounds as an example

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Abstract

The European Commission has presented a list of priority substances in addition to the Water Framework Directive (WFD) adopted in December 2000. The list of priority substances is a matter of continuous review hence other relevant substances identified as hazardous can be implemented for regulation. In that regard a group of potential hazardous substances, quaternary ammonium compounds (QAC) is selected for further investigation and assessment, as QAC are widely used as disinfectants, biocides, and detergents among a variety of other applications. This paper provides information on a general interdisciplinary approach for assessing the potential significance of chemical substances hitherto not described in a coherent way considering QACs as example.

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. For basic environmental risk evaluation, QAC usage pattern, emissions from single source polluters, the fate in waste water treatment plants, concentrations in surface water and sediments, as well as ecotoxicological effective concentrations were analyzed in this study. Based on substrate characteristics and use pattern relevant single source polluters were identified and emission concentrations as well as loads discharged into the sewerage were determined. Effluents from hospitals and laundries but also from wellness resorts showed high effluent concentrations compared to municipal waste water. To describe the fate of QACs during waste water treatment,

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adsorption and degradation behavior were determined. Additionally the influence of QACs on biological processes, especially nitrification was assessed. Partition coefficient values ($\log k_{oc}$) for QACs were determined between 4.35 for DDAC-C10 and 5.69 for DDAC-C18 (data not shown) indicating the high adsorption potential of those substances to the activated sludge in the waste water treatment plants. Results for BAC-C12–18 were found to be in the same range. Concentrations for nitrification inhibition in waste water treatment plants lay above concentrations found in municipal waste water but in the range of concentrations discharged by single source polluters.

Keywords: Quaternary ammonium compounds (QAC); Urban water circle; Hazardous substances; Analysis; Assessment of environmental significance

1. Introduction

Within the frame of the European Water Framework Directive (WFD) which was adopted in December 2000 (2000/60/EC), the European Commission (EC) agreed on a first list of priority substances in November 2001 (2455/2001/EC). At present, the EC is developing proposals for further substances on the priority list which ought to be identified and regulated as hazardous (Priority Substances Daughter Directive). There are still many chemicals, which fulfill the criteria of the EC 2003 Technical Guidance Document on Risk Assessment with respect to their wide use and hazardous effects and thus have a potential to be added to the priority list.

Quaternary ammonium compounds (QAC) form an economically important class of industrial chemicals. Because of their strong cationic surface activity, QAC are primarily used as disinfectants, biocides or preservatives and detergents, but also as anti-electrostatics and phase transfer catalysts in a wide range of applications such as fabric softeners, hair conditioners, emulsifying agents and constituents of room deodorizers, and sanitizers among others.

Madsen [1] 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 waste water treatment plants (WWTPs) before potential discharge into the environment. In the aquatic environment, QAC are characterized by a high potential to

adsorb to organic or inorganic surfaces such as suspended solids and sediments. Due to their biocide properties, they may pose a threat to either organisms in activated sludge in WWTPs [5] or in natural surface waters [11]. Therefore, QAC are selected for further investigation and assessment.

The aim of this study was to work out a comprehensive basis for environmental risk evaluation for QAC in Austrian waters. This was done in a modular study design including (a) the analysis of the sources and fate during waste water treatment, (b) surface water and sediment monitoring for exposure characterization, and (c) ecotoxicological effect characterization. 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.

2. Materials and methods

2.1. General approach

A multi step approach was chosen to assess environmental relevance for QACs (see Fig. 1). As QACs form a very heterogeneous group, the first step was to encounter the ecotoxicologically most relevant QACs. This selection was based on information provided by the manufacturers in questionnaires and retrieved from the literature. Based on that information, use pattern and relevant single source polluters were identified. A general survey on municipal waste water treatment

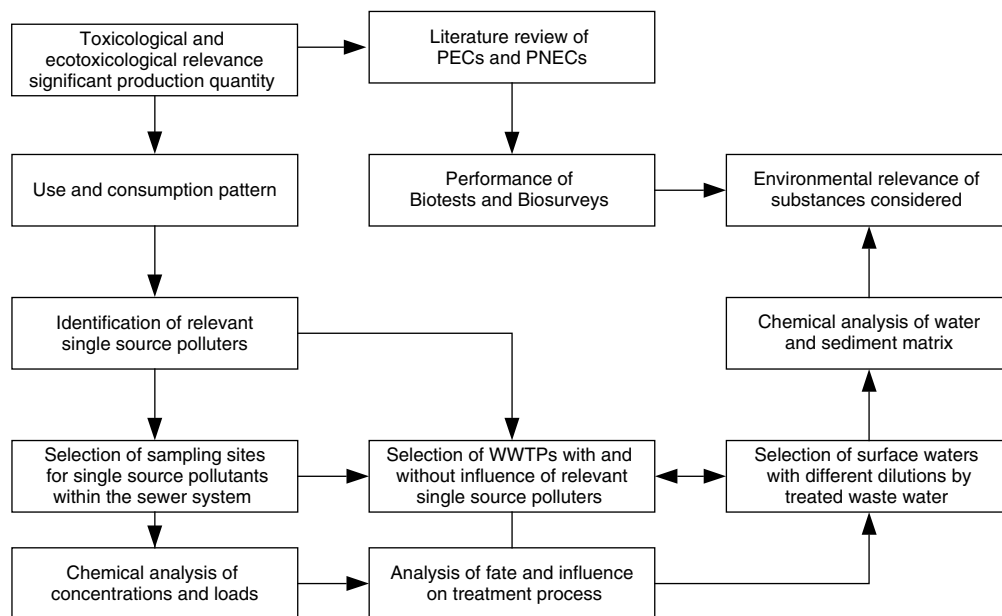


Fig. 1. Flow scheme for the general approach chosen in this study.

systems was undertaken to fill a decision matrix for further selection of the study area. Following aspects were considered:

- Sewerage with and without significant influence of single source pollutants identified as relevant for emission of target compounds.
- Different operational conditions of WWTPs in regard to sludge retention time corresponding to biological treatment applied: C removal only; nitrification; denitrification.
- Different dilution situations of surface water flow by treated waste water.

The fate of the substances within waste water treatment was accessed by mass balances [2]. Adsorption characterization necessary for mass balances were measured within the study (data not shown). Additionally the effect of QACs on the biological processes (carbon removal and nitrification) within biological waste water treatment was assessed. Loads from single source pollutants connected to the sewerage were compared to the inflow load of the respective

WWTP to evaluate their relevance by comparing those two loads.

Receiving waters linked to the WWTPs investigated and representing different dilutions by waste water were monitored as a base for ERA. Ecotoxicological effect characterization was based on both experimental determination of effective concentrations employing standardized biotest procedures for a set of representative aquatic organisms and, an extensive review and evaluation of the published original literature.

Further, analytic methods were developed and validated for different matrices (surface waters, sediments, raw waste water, activated sludge) for the determination of these selected compounds based on LC-MS/MS instrumentation, to ensure quantification and detection limits in the low ng/L and/or $\mu\text{g}/\text{kg}$ range. Overall 89 samples from different sites were analyzed in this study.

This schematic approach is performed by an interdisciplinary consortium represented by the authors with partners specialized on particulate aspects within the approach.

2.2. Selection and description of substances

Within the group of QACs benzalkonium chlorides (BAC-C12 CAS-Nr 139-07-1, BAC-C14 CAS-Nr 139-08-2, BAC-C16 CAS-Nr 122-18-9, BAC-C18 CAS-Nr 122-19-0) and dialkyldimethylammonium chlorides (DDAC-C10 CAS-Nr 7173-51-5, DDAC-C12 CAS-Nr 3401-74-9, DDAC-C14 CAS-Nr 10108-91-5, DDAC-C16 CAS-Nr 1812-53-9, DDAC-C18 CAS-Nr 107-64-2) were chosen in this study (Fig. 2).

For the quantification of QACs in the analytical process single compounds standards purchased from Aldrich and Fluka were used.

For toxicity tests a BAC mixture (ca. 60% BAC-C12, 40% BAC-C14, <1% BAC-C16; purity \geq 95%; CAS Nr 8001-54-5; Sigma–Aldrich) and dimethyl-ditetradecylammonium bromide (DDAB-C14; purity \geq 97%; CAS Nr 68105-02-2; Fluka) was used.

2.3. Chemical analysis

Water samples were characterized for BAC-C12 to BAC-C18 and DDAC-C10 to DDAC-C18 with a procedure similar to an ion-pair extraction according to [3]. 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 deionised water, evaporated and the residue dissolved in methanol. The liquid chromatographic system used for separation was an Agilent 1100 HPLC, Luna C18 (150 × 2 mm (length × i.d.), 5 μ m particle, Phenomenex Vienna). Mobile phases were 95:5 acetonitrile/water with 10 mM ammonium acetate (a), 20:80 acetonitrile/water with 1% acetic acid (b) and isopropanol with

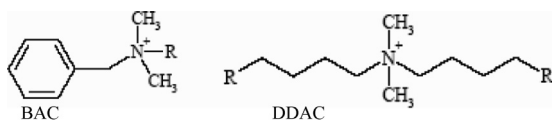


Fig. 2. Chemical structure of BAC and DDAC.

0.1% formic acid (c). A Quattro Ultima triple quadrupole mass spectrometer equipped with the electrospray interface from Micromass (Manchester, UK) was employed. Chemical analyses of QAC were conducted at the Umweltbundesamt Vienna (Spittelauer Laende 5, A-1090, Austria). The achieved limits of detection (LOD) and limits of quantification (LOQ) are given with the results.

Sediment and sludge sample from activated sludge plants were extracted (Soxhlet extraction) with MeOH acidified with approximately 1% (v/v) of fuming HCl for 18 h. The resulting extract was concentrated to 10–20 mL and afterwards evaporated to dryness under nitrogen. The residue was redissolved in CHCl₃ and extracted several times with milli-Q water. After phase separation, the solvent extract was evaporated to dryness by nitrogen and filled up with MeOH.

Detailed information on the analytical issue is published by the authors in [24,25].

Beside BAC and DDAC other parameters including standard waste water parameters were analyzed according to standardized methods [4].

2.4. Selection of single source pollutants and waste water treatment plants

Sampling points of single source pollutants were selected according to the field of application of the QAC in household, industry and small businesses (Table 1). In two cities (120,000 p.e. and 2 millions p.e.) samples were collected directly at the connecting pipes into the public sewer system from hospitals, laundries, dairies and wellness centers, but also pure household areas were sampled. One study area with five single source sampling points was located in an urban sewer system connected to a municipal WWTP (120,000 p.e.; Site 1 with mainly industrial emitters). Due to the probable use of QAC in swimming pools, waste water of a small community with a high number of swimming pools was analyzed (Site 6). Nineteen samples of single source pollutants were taken.

Table 1
Single source polluters sampled at different study sites

	WWTP 1	WWTP 2	WWTP 3	WWTP 4	WWTP 5
Wellness		X			
Dairy (1)	X				
Dairy (3)			X		
Municipal WW (1)	X				
Swimming hall (1)	X				
Paper production (1)	X				
Rendering plant					
Hospital (1)	X				
Hospital (2)		X			
Hospital (WW Oncology) (2)		X			
Laundry (1) June 2004	X				
Laundry (1) August 2004 effluent	X				
Laundry (1) Industrial cloth part after membrane filtration (Aug. 2004)	X				
Laundry (1) Hospital cloth part (Aug. 2004)	X				
Laundry (2)		X			

The investigations on waste water treatment were carried out in two perspectives: monitoring sources and the fate of QAC in WWTPs. In addition, influent, effluent and sewage sludge samples were taken from five municipal WWTPs (WWTP 1–5, Table 2) which were selected according to their location on receiving waters of different size, relevant single source emitters

Table 2
Description of waste water treatment plants (WWTPs) sampled

WWTP	Size (PE)	Characteristics
WWTP 1	120,000	PS, AB, AD C, P, N, DN
WWTP 2	2,500,000	PS, AB C, P
WWTP 3	95,000	PS, AB, AD C, P, N, DN
WWTP 4	300,000	PS, AB C, P, N
WWTP 5	230,000	PS, AB, AD C, P, N, DN

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

and plant layout. For WWTPs filtered and total unfiltered concentrations of target substances were analyzed. Forty WWTP inflow and effluent samples were taken.

Two sampling campaigns were done for both, single source polluters and WWTPs.

In general, flow-proportional 24-h composite samples were taken from the various sampling points, in few cases grab samples were collected from waste water tanks. Samples from single source polluters were taken simultaneously at the different sampling sites, the same time as the influent and effluent of the connected WWTP. The samples were stabilized with sodium azide and chloroform and stored in the fridge.

Grab samples of activated sludge samples from WWTPs investigated were collected and lyophilized before analysis.

For calculation of the emitted amount, waste water flow of single point polluters was measured with the help of bubbler probes or was assessed by the pumping intervals of pump stations. Flow from WWTPs was obtained from the process control systems of the plants.

2.5. Surface waters and sediments

Grab samples of surface water sites 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. Twenty-two surface water samples were taken from 4 receiving waters (upstream and downstream WWTP each).

Together with the surface water samples, corresponding sediment samples were taken at the same locations. They were dried at 30°C and sieved below 0.63 µm. Twenty-one sediment samples were taken.

In June 2004 surface waters showed higher than average discharge and high turbidity after rainfalls during 2 days before sampling.

2.6. Assessment of influence on nitrification in waste water treatment plants

In order to increase the knowledge on the behavior of BAC and DDAC in the process of the waste water purification, its influence on the carbon degradation and the nitrification was examined. Due to the sensitivity of nitrifying bacteria a negative influence of existing influent concentration cannot be excluded. The inhibition was tested in short term and long term tests for acute and chronic inhibition.

For acute tests oxygen consumption for carbon removal (OVC) and nitrification (OVN) were measured after addition of various concentrations (0.02, 0.2, 0.5, 1, 5, 10, 25, 50, 100 mg/L) of the single substances (BAC-C12–16 and DDAC-C10–18) according to [23].

The chronic tests were investigated in laboratory size WWTP with continuous dosage of constant concentrations and quantities of the test substances and artificial waste water (per 1000 mL: 160 mg peptone, 110 mg meat extract, 30 mg urea, 7 mg NaCl, 4 mg CaCl₂ · 2H₂O, 2 mg MgSO₄ · 7H₂O, 28 mg K₂HPO₄) as substrate

for a duration of 6 months applying final concentrations of 0.1, 1 and 2 mg/L QAC mixture described above for at least 1 month. Chronical effects were accessed by comparing C removal and nitrification efficiency with a second lab scale plant as control receiving the same inflow and operated the same way but without QAC addition.

2.7. Single species 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 population growth and reproduction were tested for green algae (*Pseudokirchneriella subcapitata*, 72-h EC50), ciliate protists (*Tetrahymena thermophila*, 24-h EC50), and rotifers (*Brachionus calyciflorus*, 48-h EC50). Daphnids were tested for acute immobilization (*Daphnia magna*, 48-h EC50). Test protocols followed established guidelines (e.g., OECD). Effect concentrations were calculated for both nominal and actual concentrations (mean recovery was 78.3% for BAC and 34.5% for DDAB). Median effective concentration values (EC50) were calculated using probit analysis.

Biotests were conducted at the University of Veterinary Medicine of Vienna (Department of Natural Sciences, Aquatic Ecotoxicology).

3. Results and discussion

3.1. Single source polluters

In order to determine the sources of these compounds and their occurrence in the aquatic environment single points of emissions into the sewer were investigated and showed, that substantial concentrations are only released from a few points. The results of the raw waste water samples from different industrial and household single source polluters are presented in Tables 3 and 4. Firstly, it can be seen, that not all of the compounds are detected in the same order of

Table 3
Results of DDAC investigation of single source waste water samples (µg/L)

	DDAC-C10	DDAC-C12	DDAC-C14	DDAC-C16	DDAC-C18
Wellness	18	<LOQ	0.011	0.021	0.12
Dairy (1)	0.014	<LOD	<LOD	<LOD	<LOD
Dairy (3)	0.29	<LOD	<LOD	<LOQ	<LOQ
Municipal WW (1)	0.30	<LOD	<LOD	0.039	0.026
Swimming hall (1)	0.017	<LOD	<LOD	<LOD	<LOD
Paper production (1)	0.094	0.081	0.015	0.023	0.12
Rendering plant	<LOD	<LOD	<LOD	<LOD	<LOD
Hospital (1)	210	<0.1	<0.1	<0.1	0.35
Hospital (2)	10	<0.06	<0.06	<0.11	0.2
Hospital (WW Oncology) (2)	0.64	0.045	0.12	0.23	0.97
Laundry (1) June 2004	64	88	7.7	6.9	9.5
Laundry (1) August 2004 effluent	44	29	4.3	1.8	3.2
Laundry (1) Industrial cloth part after membrane filtration (Aug. 2004)	87	2.2	<0.22	1.1	4.3
Laundry (1) Hospital cloth part (Aug. 2004)	82	1.8	<0.11	0.65	2.7
Laundry (2)	8.1	0.35	<0.11	0.40	1.5
LOD	0.006	0.006	0.006	0.006	0.011
LOQ	0.012	0.011	0.011	0.011	0.022

Table 4
Results of BAC investigation of single source waste water samples (µg/L)

	BAC-C12	BAC-C14	BAC-C16	BAC-C18
Wellness	46	15	0.19	0.028
Dairy (1)	1.4	0.62	0.012	0.025
Dairy (3)	20	4.6	0.12	<LOD
Municipal WW (1)	4.1	1.5	0.015	<LOQ
Swimming hall (1)	0.11	0.031	<LOD	<LOD
Paper production (1)	2.0	0.51	0.048	0.026
Rendering plant	<LOQ	<LOD	<LOD	<LOD
Hospital (1)	2800	1100	27	1.5
Hospital (2)	1140	480	27	2.4
Hospital (WW Oncology) (2)	0.63	0.39	0.013	0.025
Laundry (1) June 2004	2100	620	21	11
Laundry (1) August 2004 effluent	620	230	10	2.4
Laundry (1) Industrial cloth part after membrane filtration (Aug. 2004)	160	63	2.0	0.11
Laundry (1) Hospital cloth part (Aug. 2004)	120	50	1.5	0.81
Laundry (2)	69	24	2.1	1.3
LOD	0.006	0.006	0.006	0.006
LOQ	0.012	0.012	0.012	0.011

magnitude. BAC-C12 and DDAC-C10 were determined with the highest levels. Secondly, hospital and laundry effluents contributed the highest concentrations of BAC-C12 and DDAC-C10, with maxima of 2800 and 210 µg/L; respectively. Most of the other emitters as dairies, wellness centers as well as waste water of a small community with a high amount of swimming pools showed comparable low concentrations.

Two types of indirect dischargers proved to be highly contaminated: waste water from hospitals and laundries. In both of the selected laundries hospital linen was washed. Therefore, it was suspected that the QAC are introduced either by the linen or by the detergents. Although the BAC pattern of the hospital and laundry samples is comparable there is a clear difference in DDAC profile, which supports the assumption that the QAC are discharged by detergents.

3.2. Waste water treatment plants

In addition to the point polluters, influent and effluent samples of municipal treatment plants are investigated. Unfiltered influent samples showed maximum concentrations for BAC-C12 and DDAC-C10 with 170 and 30 µg/L, respectively (data not shown).

In the effluent of the treatment plants highest concentrations were observed for BAC-C12 and DDAC-C10 with 4.1 and 0.85 µg/L respectively (Tables 5 and 6). Mean TSS concentration in the effluents of plants were 15.3 mg/L in June and 3.25 mg/L in September. As total unfiltered effluent samples were analyzed and the QACs being highly adsorptive, the higher QAC effluent concentrations of WWTP 2 during rain weather in June can be attributed to loss of TSS.

The investigations in the WWTPs indicate a high removal rate from the waste water. Monitoring of the corresponding sludge samples indicate a rapid biodegradation of QAC in WWTPs. Removal of BAC and DDAC during sewage treatment is greater than 98%, as determined under practical conditions.

3.3. Activated sludge inhibition tests

Investigations of the inhibition of the sludge showed acute effects for OVC and OVN only at concentrations higher than 10 mg/L. This concentration causes an inhibition of the nitrification, especially of the second step of the ammonium oxidation, in which NO₂ is oxidized to NO₃. An influence on the denitrification could not be determined. Increase of NO₂ concentration

Table 5
Results for DDAC in WWTP effluents — June/September 2004 (µg/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/n.a.	0.012/n.a.	<LOQ/n.a.	0.017/n.a.	0.054/n.a.
WWTP 4	0.025/n.a.	<LOD/n.a.	<LOD/n.a.	<LOQ/n.a.	0.028/n.a.
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

n.a.: not analysed.

was observed starting from an addition of 2 mg/L. Our results are in line with those of [5], which seized an influence on the respiration likewise only with concentrations of 10 mg/L. For DDAC-C12 an influence on basic carbon respiration could be determined only starting from a dosage of 50 mg/L. The comparison between the two groups of substances of BAC and DDAC showed that the BAC was more effective than DDAC. The acute inhibition does not represent a relevant concern for WWTPs due to the results of the respiration tests. Acute inhibitions are in the concentration ranges of 0.5–1 mg/L for BAC-C12–16 and starting from 5 mg/L for DDAC.

Chronical effects on carbon removal (OVC) could not be observed for concentrations investigated (max. 2 mg/L) over a period of 3 month in a lab scale WWTP. Chronical effects on the second step in nitrification (NO₂ oxidation) could be observed for 1 and 2 mg/L. Denitrification is not influenced up to 2 mg/L.

For a comparison the analyzed inflow concentrations of the examined WWTP are clearly below that determined effect thresholds, with max. influent concentrations of 170 µg/L for BAC-C12 and 41 µg/L for DDAC-C10.

3.4. QAC concentrations in surface waters and sediments

Upon release into the environment, whether to water or sediments, several biotic and abiotic fate processes act to disperse and degrade the compounds studied. Besides mixing and dispersing in water, they are subject to adsorption to suspended solids and sediment and biodegradation. Biodegradation is the key loss process in WWTPs.

In Tables 7 and 8 BAC and DDAC concentrations of surface waters and the corresponding sediments are given. The first site (L1, Y1, S1, D1) corresponds to locations upstream the discharge of WWTPs whereas the second value (L2, Y2, S2, D2) corresponds to sites downstream the WWTPs (WWTP1 to 4), 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 in water. This observation parallels the pattern of use of QAC as also detected in single source pollution samples as well as in technical mixtures commonly applied in industrial uses. The contamination in the surface waters is not evenly distributed. One river was highly contaminated (L1 and L2) in terms of water and also sediment samples. For the other sampling sites

Table 6
Results for BAC in WWTP effluents — June/September 2004 (µg/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/n.a.	1.6/n.a.	0.69/n.a.	0.048/n.a.
WWTP 4	0.081/n.a.	0.026/n.a.	0.038/n.a.	<LOQ/n.a.
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

n.a.: not analysed.

Table 7
Results for DDAC measurements in surface waters ($\mu\text{g/L}$) and sediments ($\mu\text{g/kg dm}$) — June 2004

	DDAC-C10	DDAC-C12	DDAC-C14	DDAC-C16	DDAC-C18
	Water/sediment	Water/sediment	Water/sediment	Water/sediment	Water/sediment
L1	0.15/370	0.022/49	<LOD/<LOQ	0.05/120	0.083/370
L2	0.081/510	0.013/13	<LOD/<LOQ	0.044/9	0.077/LOQ
Y1	0.033/12	0.011/<LOD	<LOD/<LOQ	<LOQ/11	0.022/53
Y2	0.046/21	0.019/<LOD	<LOD/<LOQ	0.018/8	0.022/40
S1	0.031/<LOQ	<LOQ/<LOD	<LOD/<LOQ	<LOD/12	<LOQ/73
S2	0.069/12	0.015/<LOD	<LOD/<LOQ	0.05/140	0.19/690
D1	0.022/16	<LOD/6	<LOD/10	<LOQ/590	<LOQ/2100
D2	0.015/47	<LOD/1	<LOD/<LOQ	<LOD/82	<LOD/330
LOD	0.006/0.2	0.006/0.2	0.006/0.3	0.006/0.5	0.011/1.0
LOQ	0.012/0.7	0.011/0.7	0.011/1.1	0.011/1.7	0.022/3.5

the concentration levels of the single substances were determined well below $1 \mu\text{g/L}$, mainly below $0.1 \mu\text{g/L}$. Nevertheless, for DDAC-C10 the influence of the WWTP effluent can be detected by an increase of the river water concentrations. This is also reflected in the sediments. In the sediment samples the concentrations were between LOQ ($<1 \mu\text{g/kg dm}$) up to 3.6 mg/kg dm with high concentrations of BAC-C12 and DDAC-C18. For the Danube sample (D1) upstream of the

WWTP very high concentrations of DDAC-C18 could be detected, although the concentrations in the water was <LOQ. As DDAC-C18 is less water soluble than DDAC-C10, the adsorption to sediments is more likely.

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,

Table 8
Results for BAC measurements in surface waters ($\mu\text{g/L}$) and sediments ($\mu\text{g/kg dm}$) — June 2004

	BAC-C12	BAC-C14	BAC-C16	BAC-C18
	Water/sediment	Water/sediment	Water/sediment	Water/sediment
L1	1.9/3600	0.51/1600	0.11/350	0.094/290
L2	0.097/3000	0.074/780	0.1/150	0.086/140
Y1	0.027/60	0.012/20	0.012/3	0.014/2
Y2	0.025/22	0.024/12	0.016/3	0.019/4
S1	0.048/6	0.021/<LOD	0.012/1	0.014/<LOQ
S2	0.29/33	0.1/18	0.061/6	0.066/7
D1	0.036/86	<LOQ/63	<LOQ/17	<LOD/10
D2	0.067/150	0.026/62	<LOQ/10	<LOQ/8
LOD	0.006/0.3	0.006/0.3	0.006/0.2	0.006/0.5
LOQ	0.012/1.1	0.012/1.1	0.012/0.8	0.012/1.8

where in most cases upstream concentrations of QACs < LOQ are observed, while downstream of WWTPs concentrations > LOQ were analyzed.

The surface water concentration of cationic detergents are below the concentrations found in literature [6,7], but for investigation they applied the disulfon blue method and found concentrations in the range of 4–42 µg/L. Ferrer and Furlong [8] analyzed four sediments by means of LC-MS on BAC-C12, BAC-C14 and BAC-C16. In this study the concentrations ranged from 21 to 260 µg/kg. Fernández et al. [9] found 30 mg/kg of DDAC in a sediment sample of the Mediterranean Sea.

3.5. Effect concentrations from single compound single-species biotests

The ranges of nominal and actual effective concentrations (EC) of the BAC and DDAB compounds tested in this study are shown in Fig. 3 and reveal the same pattern of species sensitivities ranking from the most sensitive algae (*P. subcapitata*, 72-h EC50: 41 µg BAC/L, 21 µg DDAB/L) and daphnids (*D. magna* 48-h IC50: 41 µg BAC/L, 23 µg DDAB/L), the rotifers (*B. calyciflorus*, 48-h EC50: 125 µg BAC/L, 25 µg DDAB/L) to the least sensitive protozoans (*T. thermophila* 24-h EC50: 2941 µg BAC/L, 4427 µg DDAB/L; all these values are based on nominal concentrations). The two test compounds showed almost equal EC levels.

Overall, results derived from the own experiments were consistent with those reported in the literature though located at the lower range of variation reported so far. For DDAC, slightly lower nominal effective concentrations were reported from tests with the wood preservative Bardac 2280 (containing 80% DDAC as the principal active ingredient, 10% ethanol, 7–10% water, and <1% amine chloride; measured concentration of DDAC was on average 15% higher than the nominal concentration of DDAC) [10,11].

For Bardac 2280 (all nominal concentrations), the most sensitive test organisms were white sturgeon (42-day old, *Acipenser transmontanus*) with the 96-h LC50 2.5 µg/L. The 96-h LC50 values for other fish species tested ranged from 300 µg/L for fathead minnow (7-day old, *Pimephales promelas*) to 2000 µg/L for starry flounder (juvenile, *Platichthys stellatus*). Sublethal effects were observed as an increase in plasma lactate in juvenile starry flounder after 24-h exposure to 50% of the 96-h LC50 value and swimming performance decreased in juvenile rainbow trout after 12-h exposure to 50% of the 96-h LC50 value. For invertebrates, 48-h LC50 for *Daphnia magna*, *Mysidopsis bahia*, *Halella azteca*, and *Neomysis mercedis*, were 37, 39, 110, and 972 µg/L, respectively.

In summary, short term effective concentrations of BAC and DDAC or DDAB to aquatic organisms ranged over two orders of magnitude from 1 to 100 µg/L. It is noteworthy that the database on the ecotoxicity of QAC is still fragmentary, especially for longer term exposure effects of on reproduction, and development and growth of aquatic organisms as well as with respect to possible interactions with other chemicals and bioavailability when absorbed to sediment or suspended particles. In addition, our review revealed considerable deficiencies in the reproducibility of the results reported in the literature.

3.6. Risk evaluation

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.

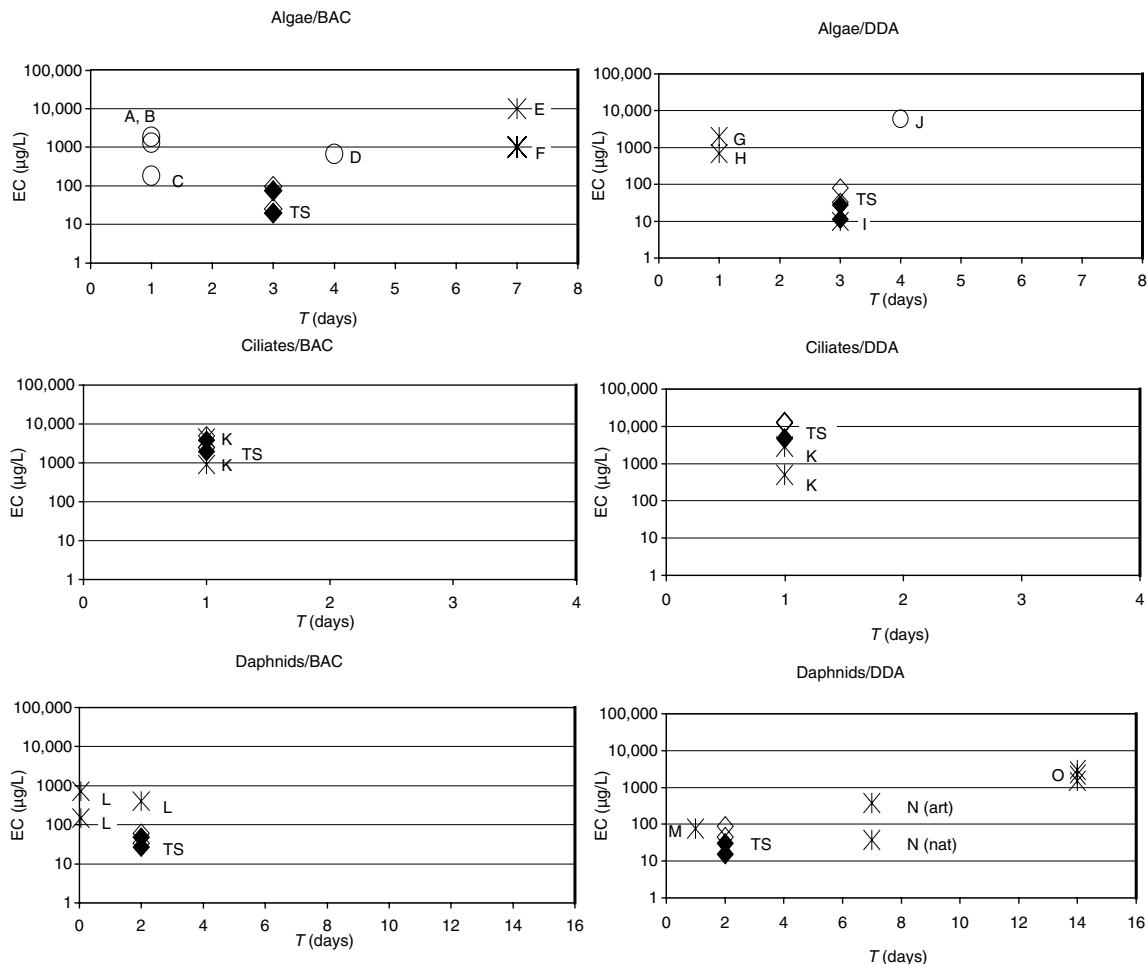


Fig. 3. Ecotoxicity of BAC and DDAC compounds (DDA, chlorinated or brominated) to algae (Chlorophyta and Cyanobacteria) and ciliate protists (population growth inhibition test), and daphnids (lethality or immobilisation tests). Effective concentrations (EC, log scaled) from the single species tests are shown relative to the duration of exposure (T). Results from this study (TS; rhombs in black for actual, in white for nominal range of test concentrations) are presented together with data from the literature with some information on alkyl chain length being available (circles) or missing (asterisks).

Abbreviations: TS: EC50 range, *Pseudokirchneriella subcapitata*, *Tetrahymena thermophila*, *Daphnia magna* [this study]; A, B, C: EC50, BAC-C12–14 (97.5%), *Dunaliella* sp. [13–15]; D: EC50, BAC C12–14 (97.5%), *Chlorella pyrenoidosa* [14]; E: EC100, *Achnanthes* sp. [16]; F: EC100, *Amphora coffeaeformis*, *Melosira nummuloides*, *Navicula hanseni*, *Navicula* sp., *Nitzschia incrustans*, *Ophephora* sp. [16]; G: EC100, *Chlorella vulgaris* [17]; H: EC100, *Stigeoclonium* sp. [17]; I: EC100, *Chlorella* sp., [18]; J: EC50, C16–18 (97.5%), *Dunaliella* sp. [15]; K: EC50, *Colpoda aspera* [19]; L: EC50, *Daphnia magna* [20]; M: EC50, *Ceriodaphnia dubia* [21]; N: NOEC; artificial (art) or natural (nat) dilution water; *Ceriodaphnia dubia* [22]; O: LOEC, EC50, NOEC, *Daphnia magna* [22].

It needs to be stressed out, that the lowest actual ecotoxicological effective concentrations determined exceeded the highest concentrations measured in surface waters (BAC-C12 1.9 µg/L; DDAC-C10 0.15 µg/L) and effluents (BAC-C12 4.1 µg/L; DDAC-C10 0.85 µg/L) only by 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. For risk evaluation, the PEC/PNEC ratio was above 1 for one river (L1) with low discharge and high content of treated waste water which indicates a potential risk for the aquatic environment. Hence for the Austrian sites studied, QAC-derived threat to sensitive aquatic non-target organisms could not be excluded.

Unlike the surface water sample the sediment concentrations are well below the PNEC value [12] for DDAC-C10 and DDAC-C18 of 15 and 55 mg/kg respectively.

4. Conclusion

The available database for QAC environmental risk assessment is still fragmentary, therefore, high assessment factors were necessary for calculation of PNEC values. The investigations of the waste water samples indicated, that the concentrations are below the effect concentrations for microorganisms in WWTPs. The pollution sources could be related to two single source pollutants: hospitals and laundries. For surface water, environmental risk could not be excluded in the risk evaluation, as the PEC/PNEC ratio was above 1 for one river (L1) with low discharge and high content of treated waste water. The sediment concentrations are well below the PNEC values.

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