Swimming pool water—fractionation and genotoxicological characterization of organic constituents

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Abstract

Swimming pool water treatment in general includes flocculation, sand filtration, and subsequent disinfection with chlorine. The continuous chlorination and input of organic material by bathers in combination with recirculation of the pool water leads to an accumulation of disinfection by-products (DBPs) in the water. Several DBPs have been identified as human carcinogens and are thought to cause allergic asthma. Therefore, the elimination of DBPs is one major aim of pool water treatment. Using membrane filtration as an alternative treatment technology, DBPs can be removed more efficiently than with conventional treatment. In this study membrane filtration and genotoxicity testing were applied for the characterization of pool water constituents and for the identification of the necessary molecular weight cut off of the membrane for an efficient elimination. Two-step membrane filtration revealed that most of the DBPs (as adsorbable organically bound halogen, AOX) were present in the molecular weight fraction below 1000 g/mol. The fraction below 200 g/mol contained more than 30\% of the AOX. The distribution of the dissolved organic carbon (DOC) across the fractions was similar to that of the AOX. The genotoxicity was found to be strongest in the low-molecular weight fraction. Thus, considerable DBP removal by membrane treatment requires membranes with low-molecular weight cut offs down to 200 g/mol. The comprehensive elimination of the genotoxic compounds requires further treatment steps.

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1. Introduction

Hygienic safety is of utmost concern for the quality of swimming pool water. This is illustrated by a number of outbreaks of diseases which were caused by micro-organisms in pool water (Leoni et al., 1999; Friedman et al., 1999). A disinfection step in pool water treatment is therefore well accepted. It is mostly performed by chlorination because of its fast reaction and lasting disinfection potential. Nevertheless, chlorine is known to produce disinfection by-products (DBPs) by reacting with inorganic and organic water constituents. Trihalomethanes (THMs) were the first DBPs which were identified in drinking water by Rook (1974). First reports about THMs in swimming pool water were published in the early 1980s (Lahl et al., 1981; Eichelsdörfer et al., 1981; Maierski et al., 1982; Aggazzotti and Predieri, 1986). DBP formation in pool water is enhanced by three important factors: (1) the
recirculation of the pool water, (2) the continuous chlorination and (3) the introduction of organic material by bathers. Chu and Nieuwenhuijzen (2002) reported that concentrations of the total organic carbon (TOC) and of THMs correlated well with the overall bather number in a pool. In detail, Glauner et al. (2004) observed a 24–48 hours time-shifted formation of DBPs after a significant increase in the TOC concentration of the water. This clearly reveals a series of reactions for DBP formation taking place during repeated treatment cycles.

Swimming pool water DBPs were found to be responsible for affecting the human well-being and health (Aiking et al., 1994) due to eye and skin irritations (Wildsoet and Chiswell, 1989; Erdinger et al., 1998) and for causing allergic asthma (Thickett et al., 2002; Bernard et al., 2003). THMs are the dominant DBPs in swimming pools. They are rated as potential carcinogens which are taken up by bathers by inhalation and permeation through the skin (Fantuzzi et al., 2001; Whitaker et al., 2003). Moreover, Honer et al. (1980) showed the presence of more than one mutagen in pool water extracts by using the Salmonella/mammalian-microsome test. In terms of health care the elimination of DBPs has to be a major task of pool water treatment. Pool water treatment in Germany has reached a high standard. According to the German standard DIN 19643 four treatment combinations including adsorption, flocculation, sand filtration, oxidation, and disinfection can be used. Nevertheless, the results of a recent survey conducted by Stauder and Baldauf (2004) showed that 70% of the public pools in Germany only apply flocculation and sand filtration prior to disinfection. This results in a limited elimination efficiency for DBPs and their precursors. Moreover, the sand filter can be considered as an additional source for DBPs due to its ability to concentrate organic contaminants, which then undergo reactions with the chlorinated pool water in the filterbed (Frimmel et al., 2004). To reduce the filter load shorter backwash cycle times would be required. Due to the higher backwash water consumption this is, however, not attractive from an economical point of view.

An attractive approach is the application of membrane filtration because of its higher elimination efficiencies for DBPs and their precursors. In addition the accumulation of contaminants on the membranes is lower due to their smaller volume and the more frequent backwash cycles. At the same time an overall lower backwash water consumption can be obtained. Since the costs for membrane filtration are correlated with the transmembrane pressure which has to be applied and therefore with the membrane molecular weight cut-off (MWCO), it is essential to determine the minimum MWCO, which is required for DBP removal.

The aim of this study was to determine the molecular weight distribution of swimming pool water DBPs and its correlation linked to biological effects. Therefore, membrane fractionation of swimming pool water was combined for the first time with genotoxicity measurements in the fractions using the comet assay. If genotoxicity is prominent in a certain molecular weight fraction, a membrane with a MWCO suitable for the removal of DBPs and for genotoxic compounds can thus be selected based on the combined results from fractionation and genotoxicity testing. In detail, two-step membrane filtration was applied to samples from an indoor and outdoor pool (IP and OP). The fractions were characterized and compared by the TOC concentrations, the adsorbable organically bound halogens, and by their DBP formation potentials. A genotoxicity testing by the comet assay was applied to the fractions from the IP to identify the putative genotoxic molecular weight fraction formed during pool water treatment.

2. Materials and methods

2.1. Sample collection and onsite analysis

Samples with a volume of ten liters were collected into glass bottles from an indoor (once in March 2003, IP) and from an outdoor swimming pool (twice, in July and August 2003, OP_1 and OP_2). Both pools were operated with a treatment of flocculation—sand filtration—chlorination with chlorine gas. Filling water for both pools was tap water, which was different for the IP and the OP. Sodium thiosulfate was added to the IP sample to scavenge the residual chlorine, but it was not added to the OP samples since maximum formation potentials were measured for these samples. For THM analysis, additional samples were collected in 40 mL vials, which were filled to the top and were tightly sealed with screw caps. Free and total chlorine were measured photometrically at the pool site with a chlorine cell test (Merck, Darmstadt, Germany) after reaction with N,N-dipropyl-p-phenylenediamine (DPD) according to German Standard DIN 38408 Part 4 (1984). The pH and electrical conductivity were measured using electrodes from WTW (WTW LF 318, WTW pH 325, Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). Samples from the OP were numbered chronologically. The experimental setup for the fractionation of each sample and the performed measurements are listed in Table 1.

2.2. Laboratory analysis

Pool water samples and fractions from the membrane filtration were characterized by TOC and adsorbable organic halogens (AOX). TOC was measured with a TOC-analyzer Sievers 820 PMT (Ionics instruments, Boulder, Colorado USA), AOX were measured with a
TOX analyzer Euroglas ECS 1200 (Thermo Electron GmbH, Dreieich, Germany) according to EN 1485, 1996.

THMs were analyzed by purge and trap sampling, capillary gas chromatography with electron capture detection (Chrompack CP 9000, Varian Inc., Palo Alto, USA) according to ISO 10301. The molar concentrations of all THMs measured were transformed to a mass concentration with the molecular weight of trichloromethane. Results are given as total trihalogenmethanes (TTHM).

Maximum formation potentials of TTHM (TTHM–FP) and of AOX (AOX–FP) were determined for samples of the outdoor swimming pool and fractions according to DVGW W295 (1997). Briefly samples were fortified with chlorine (20 mg/L) and reacted 48 h during shaking at room temperature. Subsequently, sodium thiosulphate was added to scavenge the residual chlorine, and THM and AOX was measured. Before AOX measurement volatile compounds were stripped with nitrogen for 10 min.

Finally, liquid–liquid extractions were performed according to Mayer et al. (1994), to produce extracts of the IP fractions for genotoxicity measurements. Briefly, 200 g sodium chloride (baked at 220 °C, 12 h) were added to 1 L of sample, which was then extracted with 25 mL methyl tertiary butyl ether (MTBE). The organic extract was blown down with nitrogen to 100 µL. Four individual extracts were produced for each fraction.

2.3. Fractionation procedure

The fractionation procedure was adapted from a multi-stage ultrafiltration which was used for the fractionation of the natural organic matter from a brown water lake and which resulted in several distinct molecular weight fractions (Müller et al., 2004). The fractionations of the swimming pool waters were performed in a 2-stage membrane filtration procedure. An ultrafiltration membrane made from regenerated cellulose (type YM, Millipore) with a molecular weight cut off of about 1000 g/mol and a nanofiltration membrane made from polyamide (type NF200-400, DOW Filmtec) with a molecular weight cut off of about 200 g/mol were used in stirred cells to obtain three fractions. Fig. 1 illustrates the fractionation procedure and Table 2 summarizes the main operating parameters. Each fractionation step was finished after reaching a concentrate volume of about 0.1 L. Resulting filtration times are listed in Table 3. After the filtration with the UF membrane the concentrate (F1) and the permeate were both diluted to a final volume of 6 L. Fractions after the nanofiltration F2 and F3 were treated in the same way.

All membranes were rinsed with ultrapure water (MilliQ, Millipore) prior to use until no further DOC release was observed. DOC and AOX mass balances were calculated to determine the recovery of each fractionation procedure.

2.4. Molecular weight cut off (MWCO) determination

The molecular weight cut off and the pore size distribution of the membranes used were determined.
according to Cleveland et al. (2002). Fractionation conditions were the same as for the samples. Different organic compounds including glycerin (116 g/mol), maltose (361 g/mol), raffinose (504 g/mol) and polyethylene glycols with molecular weights of 200, 1550, and 6000 g/mol were used as calibration standards. Solutions of the compounds in ultrapure water with a TOC concentration of about 5 mg/L were filtered successively starting with the compound with the lowest molar mass. In case a TOC retention of more than 10% was observed for one compound, the membrane was replaced with a new one prior to the filtration of the next compound. All membranes were rinsed prior to use with ultrapure water until no further DOC release was observed.

2.5. Genotoxicity assay using the comet-assay

The Comet assay was performed according to Singh et al. (1988), with slight modifications. Hep-G2 cells (German National Resource Center for Biological Material, DSMZ) were exposed on six-well microplates in 3 mL RPMI 1640 medium (Sigma, Deisenhofen, Germany) spiked with MTBE as negative control sample, the MTBE extracts of the pool water fractions F1, F2, and F3 (15 µL extract/3 mL medium and 30 µL/3 mL medium) or with the genotoxic agent 4-nitroquinoline-1-oxide (NQO) as positive control, for 2 h (37°C, 5% CO2, 50 rpm). After treatment the cells were washed twice with 3 mL of phosphate buffered saline (PBS) and detached with 100 µL of trypsin (0.1% in PBS, without Ca2+ and Mg2+, 0.02% EDTA, 6 min, 37°C). Enzymatic digestion was stopped by adding 100 µL medium with 40% fetal bovine serum (FBS). Then, 15 µL of the cell suspension were mixed with 90 µL of 0.7% low melting agar (LMA, Biozym, Hess. Oldendorf, Germany) and pipetted onto a slide precoated with a layer of 1% normal melting agar (NMA, Biozym, Hess. Oldendorf, Germany). After solidification on ice, another layer of 90 µL LMA was added and the slides were immersed into lysis solution (2.5 M NaCl, 10 mM Tris, 100 mM EDTA, NaOH of pH 10.0, 1% Na-sarcosinate, 10% dimethylsulfoxide and 1% triton X-100) for 1 h. The slides were then incubated in an electrophoresis tank containing 300 mM NaOH with 1 mM EDTA for 45 min prior to electrophoresis for 35 min at 25 V (300 mA). Subsequently, the slides were neutralized (0.4 M Tris, pH 7.4), fixed in absolute ethanol for 10 min and stained with 40 µL ethidium bromide (2 µg/mL, Sigma) for fluorescence microscopy analysis using a digital imaging system (Optilas, München, Germany). The extent of the DNA damage was quantified measuring the tail moment, i.e. the integrated value of relative fluorescence intensity in the tail multiplied by the migration distance of the DNA fragments. Cells with increased DNA single-strand breaks show an increased proportion of short DNA fragments covering a longer distance from nucleus toward the anode in the electric field. In the microscopic picture these cells look like a comet with a head—the nucleus—and a tail formed by short DNA fragments. Cells with a low level of DNA strand breaks have long DNA strands and show an almost intact nucleus. For statistical analysis the median of the tail moment values of 100 cells measured per treatment was determined. Significant differences (P<0.05) from the negative control were revealed with analysis of variance (ANOVA). Cell viability was determined using a fluorescein diacetate assay according to Strauss (1991).

3. Results and discussion

3.1. Filtration characteristics of the membranes

For the identification of the molecular weight fractions of swimming pool water DBPs, obtained by membrane filtration, it is essential to know the molecular weight cut-off of the membranes. Therefore the MWCO of the membranes was estimated under the conditions used for fractionation. Fig. 2 shows the
solute rejection curves for the two different membranes based on the filtration of organic standard compounds. The curves can be used to determine the molecular weight at which 90% of the TOC is rejected ($P_{90}$). According to Lee et al. (2002) the $P_{90}$ can be defined as nominal molecular weight cut off (nMWCO) of a membrane. In addition, the slopes of the curves describe the pore size distribution of the membrane. Under the applied conditions (stirred cells, $T = 12\, ^{\circ}\mathrm{C}$, $p = 0.5\, \text{MPa}$) the nMWCO for the used NF membrane was estimated to be approximately $200\, \text{g/mol}$. For the UF membrane the nMWCO was estimated to be approximately $1000\, \text{g/mol}$. These results are in good agreement with data given by the membrane suppliers. The NF membrane showed a very narrow pore size distribution whereas the slope of the rejection curve of the UF membrane was less steep, indicating a wider pore size distribution. Therefore, in particular in fraction F1 a broader molecular weight distribution was expected to occur.

### 3.2. Membrane performance and filtration characteristics

Differences in the composition and in the amount of the organic load of the pool water samples can lead to a different behavior during the two subsequent membrane filtrations. Therefore the membrane performance during the fractionation experiments was observed. Table 3 shows the mean values for the volumetric flux, the permeability, and the filtration time of the two filtration steps for the three samples. The whole fractionation experiment took $61.5\, \text{h}$ for OP_1, about $54.7\, \text{h}$ for OP_2, and $72.8\, \text{h}$ for the sample from the IP. For the ultrafiltration stage the volumetric flux as well as the filtration time was more or less constant for all fractionation experiments, i.e. from $11.7\, \text{L h}^{-1}\, \text{m}^{-2}$ to $13.8\, \text{L h}^{-1}\, \text{m}^{-2}$ and from $22.1$ to $26.8\, \text{L h}^{-1}\, \text{m}^{-2}$, respectively. However, for the nanofiltration stage of the IP water the volumetric flux was significantly lower and the filtration time was 35% longer compared to the fractionation experiments of the OP samples. The reason for that was mainly the accumulation of thiosulfate in F2 which resulted in a higher concentration polarization and therefore in a reduced volumetric flux. This was also obvious from the decrease of the permeability. Whereas the mean permeability for the NF was $0.14\, \text{L h}^{-1}\, \text{m}^{-2}\, \text{MPa}^{-1}$, a strong decrease of the permeability during fractionation was observable. Initially, the permeability was $0.21\, \text{L h}^{-1}\, \text{m}^{-2}\, \text{MPa}^{-1}$, but at the end of the experiment ($0.1\, \text{L residual volume of the concentrate}$) the permeability was less than $0.1\, \text{L h}^{-1}\, \text{m}^{-2}\, \text{MPa}^{-1}$. Such a decrease was not observed for the OP samples to which no sodium thiosulfate was added.

#### 3.3. Chemical characterization of pool waters

The chlorine dose and the concentration of organic compounds introduced by bathers and by the filling water are the key parameters for the DBP formation in pool waters. At the time of sampling the three pool water samples showed free chlorine concentrations of $0.7\, \text{mg/L}$ for IP, $0.4\, \text{mg/L}$ for OP_1 and $0.7\, \text{mg/L}$ for OP_2. The concentrations of chloramines were $0.4\, \text{mg/L}$ for IP and $0.1\, \text{mg/L}$ for both OP samples. Table 4 shows the load of the original pool waters with TOC as well as with disinfection by-products (as AOX and TTHM). TOC concentrations of both pools were similar. As expected a higher number of bathers resulted in a higher TOC concentration in the OP. In contrast the IP showed the highest AOX concentration but lower TTHM values compared to both samples of the OP. If the FP for AOX and TTHM of the two OP samples which differ in their bather load are compared, no increase in AOX–FP is seen for OP_1 and an increase of $44\, \text{mg/L}$ is seen for OP_2. TTHM increased for both samples by $46\, \text{mg/L}$ for OP_1 and by $88\, \text{mg/L}$ for OP_2. This can be explained by the higher number of visitors, which led to more “fresh” organic material in OP_2 compared to OP_1, which reacted readily with chlorine. The pool water samples which have seen only a very low bather load, typically showed no further AOX formation, since the organic material already has had sufficient time for exhaustive reactions. On the other hand, THM formation in general requires many more reaction steps of chlorination and C–C bond cleavages. Therefore, pool water samples still show THM–FP after several days even in the case of low bather loads. In a recent study a 24 h-delayed AOX maximum and a 48 h-delayed THM maximum have been observed in an OP after days with peaking bather load (Glauner et al., 2004).
3.4. Molecular weight fractions of pool water contaminants

The identification of the molecular weight fraction accounting for the major proportion of the DBPs and their precursors is a prerequisite for the optimization of the elimination efficiency in pool water treatment. Table 4 shows the concentrations of TOC, AOX, TTHM, and of the formation potentials for AOX and TTHM in the three pool water fractions after membrane filtration. In all experiments, the TOC mass balance was between 80% and 115% and the AOX mass balance was between 60% and 105% compared to the original pool water. Due to their volatility and the open filtration system used THMs were not expected to be recovered during fractionation. The AOX–FP mass balance was 116% for OP_1 and was even 144% for OP_2. As all samples were treated with the same chlorine concentration of 20 mg/L one reason for the high yield might be the larger chlorine to TOC ratio which was applied to the fractions compared to the original sample.

Fig. 3 shows a comparison of the fractions of TOC and AOX for IP and OP_1 water. IP is dominated by fraction F2 for the TOC (54%). The AOX is mainly contained in the low-molecular weight fractions, i.e. in fraction F2 and F3 but the high-molecular weight fraction F1 contributes considerably with 18%. F3 contains 32% of the TOC and 36% of the AOX. Also in OP_1 water the low-molecular weight fractions F2 and F3 are mainly responsible for the AOX with only 6% of the AOX present in F1. This is also reflected by

<table>
<thead>
<tr>
<th>Pool site/sample</th>
<th>Date</th>
<th>Number of visitors per day</th>
<th>TOC (mg/L)</th>
<th>AOX (µg/L)</th>
<th>AOX–FP (µg/L)</th>
<th>TTHM (µg/L)</th>
<th>TTHM–FP (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP</td>
<td>03/17/03</td>
<td>—*</td>
<td>1.7</td>
<td>235 ± 15</td>
<td>n.a.***</td>
<td>21</td>
<td>n.a.***</td>
</tr>
<tr>
<td>Original sample</td>
<td>F1</td>
<td>0.2</td>
<td>41 ± 4</td>
<td>n.a.***</td>
<td>n.d.**</td>
<td>n.a.***</td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>0.7</td>
<td>99 ± 6</td>
<td>n.a.***</td>
<td>n.d.**</td>
<td>n.a.***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>0.4</td>
<td>80 ± 5</td>
<td>n.a.***</td>
<td>2</td>
<td>n.a.***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum of fractions</td>
<td></td>
<td>1.3 (76%)</td>
<td>220 (93%)</td>
<td>n.a.***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OP_1</td>
<td>07/09/03</td>
<td>818</td>
<td>1.6</td>
<td>177 ± 22</td>
<td>175 ± 4</td>
<td>35</td>
<td>81</td>
</tr>
<tr>
<td>Original sample</td>
<td>F1</td>
<td>0.5</td>
<td>11 ± 1</td>
<td>47 ± 12</td>
<td>n.d.**</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>0.6</td>
<td>106 ± 1</td>
<td>74 ± 1</td>
<td>1.2</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>0.7</td>
<td>70 ± 1</td>
<td>82 ± 6</td>
<td>7.0</td>
<td>35</td>
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<td>135 (85%)</td>
<td>212 (121%)</td>
<td>n.a.***</td>
<td>77 (95%)</td>
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<td>OP_2</td>
<td>08/06/03</td>
<td>2341</td>
<td>2.0</td>
<td>161 ± 4</td>
<td>205 ± 6</td>
<td>47</td>
<td>135</td>
</tr>
<tr>
<td>Original sample</td>
<td>F1</td>
<td>0.5</td>
<td>17 ± 1</td>
<td>40 ± 2</td>
<td>1</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>0.8</td>
<td>33 ± 1</td>
<td>96 ± 1</td>
<td>2</td>
<td>65</td>
<td></td>
<td></td>
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<td>F3</td>
<td>1.0</td>
<td>46 ± 6</td>
<td>133 ± 4</td>
<td>9</td>
<td>147</td>
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<td>2.4 (117%)</td>
<td>99 (62%)</td>
<td>177 (86%)</td>
<td>n.a.***</td>
<td>233 (173%)</td>
<td></td>
</tr>
</tbody>
</table>

*no data available; **n.d. = not detectable; ***n.a. = not analyzed.

Values given with the standard deviation of at least three individual samples. TOC values were obtained by measuring five replicates of the same sample. Relative standard deviation for TOC measurements were always below 2%.

Table 4
Characterization of pool water fractions by the parameters TOC, AOX, AOX–FP, TTHM, and TTHM–FP
the AOX–FPs. The bathers load contributed mainly to the fractions F2 and F3 since the TOC increase for OP_2 compared to OP_1 was only observed in these fractions. The input from bathers load in OPs may be explained by a much higher input of compounds from sunscreens and other personal care products compared to IPs. The TOC of the high-molecular weight fraction F1 did not increase with an increasing number of bathers and therefore could be attributed mainly to the TOC of the raw water.

3.5. Genotoxicological evaluation of the fractions

The application of the comet assay to extracts of the pool water fractions should reveal the identification of the putative genotoxic molecular weight fraction formed during pool water treatment. Table 5 shows the results of the comet assay applied to the MTBE extracts of the IP water fractions. In fraction F1 no genotoxic effect was observed at all, whereas in F2 only a low genotoxicity was observed, which showed a significant effect just at the higher concentration factor tested. A significant genotoxic effect was observed for F3 for both concentration factors. For these samples, a distinct concentration related effect could be observed. Fraction F3 contains predominantly non-volatile, low-molecular weight compounds comprising 32% of the TOC and 36% of the AOX of the original sample. One has to consider that only compounds, which are extractable by MTBE could have contributed to the genotoxic effect. For a further development and optimization of pool water treatment these genotoxicity measurements in addition to sum parameters like TOC and AOX revealed valuable information. The consequence would be that not only membrane separation is suitable to reduce DBPs from pool water. Ozonation or advanced oxidation procedures are further promising methods to remove the low-molecular weight fractions, which show the strongest genotoxic effects. The identification of genotoxic compounds and the evaluation of their mode of action are further attractive aims for future investigations as a thorough risk assessment of genotoxic compounds requires the identification of both structure and effect mechanism.

4. Conclusions

In this work pool water fractions from two different swimming pools were obtained by applying a 2-stage membrane filtration procedure. The pool water samples and the different fractions could be well characterized by different laboratory parameters comprising the TOC, AOX, and TTHM as well as the formation potentials for AOX and TTHM. For a selected sample from an IP water genotoxicological measurements of the fractions were performed by a Comet assay. The results of the experiments led to the following conclusions:

- The 2-stage membrane filtration resulted in three different fractions based on TOC and AOX measurements. Comparing the samples of the indoor (IP) and of the outdoor pool (OP_1), the high-molecular weight fraction F1 of OP_1 showed a higher proportion of the TOC, but a much lower one of the AOX. However, comparing the two outdoor pool samples for low (OP_1) and high (OP_2) numbers of visitors revealed differences mainly for the low-molecular weight fractions F2 and F3. The high-molecular weight fraction F1 is therefore assumed to be less influenced by the bather load rather than the organic material already present in the filling water. Those high molecular weight fractions can be expected to be efficiently removed by adsorption or ultrafiltration in technical water treatment.

- Genotoxicity measurements of the indoor pool sample reveal a significant effect only for fraction F3, containing non-volatile, low-molecular weight compounds. Removal of such compounds would require nanofiltration with very low-molecular weight cut offs. These membranes are practically not used for economical reasons. Ozonation or advanced oxidation processes (AOP) are the more promising alternatives in this context to eliminate or

| Table 5 |
| Genotoxicity measurements of the fractions of IP with the comet-assay after MTBE extraction |
|----------------------------------|-----------------|
| Tailmoment                       |                 |
| Negative control (15µL MTBE/3mL medium) | 2.4            |
| Negative control (30µL MTBE/3mL medium) | 3.9            |
| Positive control (0.5mM NQO)     | 118.4          |
| Extract of F1 (>1000 g/mol)      |                 |
| Concentration factor 50 (15µL MTBE/3mL medium) | 1.1            |
| Concentration factor 100 (30µL MTBE/3mL medium) | 1.8            |
| Extract of F2 (200–1000 g/mol)   |                 |
| Concentration factor 50 (15µL MTBE/3mL medium) | 6.6            |
| Concentration factor 100 (30µL MTBE/3mL medium) | 11.2*          |
| Extract of F3 (<200 g/mol)       |                 |
| Concentration factor 50 (15µL MTBE/3mL medium) | 10.5*          |
| Concentration factor 100 (30µL MTBE/3mL medium) | 33.3*          |

*Significant (P<0.05). The viability of the treated cells was >90% in all cases.
at least partially degrade the adverse effect compounds.

In this investigation the Comet assay was successfully used to get—in addition to TOC and AOX—bio-effect related information for the optimization of existent and the implementation of new treatment techniques. It has, however, to be kept in mind that risk assessment is only possible on the basis of identified single compounds which are responsible for the effect and on the knowledge of the mode of action of these substances.

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