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Haloacetonitriles –another important group of disinfection byproducts in swimming pool water

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1 Synopsis

Minimization of toxic disinfection byproducts (DBPs) in swimming pool water is a principal key issue in optimization of pool water treatment. One important parameter indicating the magnitude of DBP formation is the concentration of trihalomethanes (THMs). According to standard DIN 19643, operators of swimming pools must comply with a limit value for THMs in Germany. Because of this, most often, THMs are used as the only indicator for the large group of possibly health-endangering DBPs, which can be formed upon chlorine disinfection. However, it is well-known that formation of other DBPs does not always correlate with THM formation. An increasing public interest asks for characterization of additional DBP being toxicologically relevant and for corresponding minimization strategies. The goal of our study was to get further insight into types and characteristics of toxicologically relevant, possibly still unknown disinfection byproducts in swimming pool water. More than 300 samples collected from 11 indoor swimming pools covering different treatment technologies were assessed by both biological assays and chemical analysis via GC/MS. The data revealed a good correlation between the biological effect observed in the sample extract and its content with haloacetonitriles (HANs), especially with bromochloroacetonitrile and dibromoacetonitrile. HANs being formed via reaction of organic nitrogen compounds with chlorine are certainly one important group of DBPs in swimming pool water that need more attention in future.

2 Background

The goal of this study was to get further information about possible lead compounds, which can indicate the presence of toxic DBPs in the swimming pool water. The test strategy should indicate toxic effects of disinfection byproducts while also providing information on the identity of toxicologically relevant single compounds. An approach considering these demands combines application of a suitable biological toxicity assay and instrumental chemical analysis.

3 Methods

3.1 Sample Preparation

DBPs in swimming pool water were extracted by liquid-liquid extraction with methyl tert-butyl ether (MTBE) at neutral pH allowing for a selective enrichment of semi-volatile polar DBPs. Using this method, 1L swimming pool water were mixed with 200 g NaCl and 25 mL MTBE. The mixture was shaken for 20 min in horizontal position at 130 rpm. Subsequently, the organic phase was transferred into a GC-vial and concentrated to 100 µL via a cold nitrogen stream (concentration factor 10.000). This type of sample concentration allowed the simultaneous investigation of the extracts in a genotoxicity test and in chemical analysis

(GC/MS). In order to avoid toxic effects of the solvent, however, the extract was diluted with culture medium before use in the biotest resulting in a maximum concentration factor of 185.

3.2 Bacterial genotoxicity assay

As a short-term bacterial test system being capable of detect both genotoxic and cytotoxic potencies of complex mixtures, the *Salmonella* umu-test was applied according to ISO 13829, 2000 (1). The umu test was performed with and without metabolic activation using a rat-liver S9 fraction, the latter for detection of genotoxicants which need a metabolic activation by mammalian biotransformation processes.

Two tester strains were used:

- *Salmonella typhimurium TA1535/psk1002* is a standardized tester strain (ISO 13829), which is used for the screening of environmental mutagens and carcinogens.
- *Salmonella typhimurium TA1535/NM5004* is a derivative of strain *TA1535/psk1002*, into which the rat GSTT1-1 gene has been cloned. The *TA1535/NM5004* strain expresses a Glutathione S-transferase theta 1 enzyme (GSTT1), which provides structural and catalytic similarities to the human protein.

Glutathione S-transferases (GST) are key enzymes in biological detoxification processes. GSTT1 plays a major role in phase-II biotransformation of a number of environmental toxicants. However, GSTT1-catalyzed reactions can also increase the toxicity of some halogenated compounds, such as dichloromethane, bromodichloromethane and 1.3-dichloroacetone (2, 3). The GSTT1-transfected *Salmonella* strain was selected for investigation of DBPs in this study, because of its known sensitive reaction for halomethanes (4). By combination of both tester strains the informative value of the assay for special DBPs should be increased, allowing for investigation of a larger substance spectrum. In addition the test provides selective information on substances, which are activated particularly by the enzyme GSTT1 to genotoxicants.

3.3 GC/MS-Screening

DBPs were comprehensively identified using gas chromatography (GC) coupled to mass spectrometry (MS). Using this method, 27 selected substances of characteristic DBP groups including halogenalkanes and -alkenes, halonitromethanes, haloacetonitriles, haloamides, and halogenated aldehydes were quantified.

4. Results

4.1 High sensitivity of the GSTT1 tester strain to DBPs of swimming pool water

Investigation of single DBPs

In order to estimate the suitability of the test system particularly for swimming pool water, 48 model substances from characteristic DBP groups were studied (table 1). The GSTT1-tester strain reacted usually more sensitively than the tester strain without GSTT1 (e.g. on dihalogenmethane, 1.2-dibromo-3-chloropropane, bromoform, dibromoacetonitrile, bromochloroacetonitrile, dichloroacetic acid). An exception is dibromoacetic acid, which caused a genotoxic effect only without GSTT1. The external metabolic activation (S9-fraction) caused a reduction of the cytotoxicity with both tester strains and partly also an enlargement and/or a shift of the genotoxic concentration range. With regard to the lowest concentration that induced a significant genotoxic response, the results show that they cover a range from few micrograms to several grams per litre depending on the tested single

substance. Because these lowest effect concentrations in the umu test significantly exceed concentrations measured in the swimming pool water (table 2), pool water samples had to be concentrated prior toxicological testing.

Table 1: Effect of DBPs on two different tester strains without external metabolic activation (S9-fraction). Average values for genotoxicity and cytotoxicity determined at different test concentrations based on at least 3 independent repetitions. DBPs which showed no genotoxic or cytotoxic response at test concentrations of 5.8 – 185 mg/L (trichloroethene, tetrachloroethene, benzaldehyde, benzacetaldehyde, phenylacetonitrile, benzophenone, styrene, sodium bromate, sodium chlorate) or up to 1850 mg/L (chloroforme, chloral hydrate, chloroacetamide, dichloroacetamide, trichloroacetamide) are not listed.

DBP test substance	tested concen-tration range	tester strain TA1535/psk1002 without GSTT1 effective concentration range		tester strain TA1535/NM5004 with GSTT1 effective concentration range	
		genotoxic	cytotoxic	**genotoxic**	cytotoxic
dihalogenalkanes					
dichloromethane	5.8 - 185	negative	negative	**23.1 – 185**	negative
bromochloromethane	0.09 - 185	**185**	negative	**5.8 – 92.5**	185
1,2-dichloroethane	0.09 - 185	negative	negative	**2.9 – 185**	negative
1,2-dibromoethane	0.045 - 185	negative	negative	**0.09 – 2.9**	5.8 – 185
1-bromo-2-chloroethane	0.045 - 185	negative	negative	**0.09 – 5.8**	11.6 - 185
trihalogenalkanes					
bromoform	58 – 1850	negative	952 - 1850	**231 – 463**	925 – 1850
bromodichloromethane	58 – 1850	**231 - 463**	952 - 1850	**231 – 925**	1850
dibromochloromethane	58 – 1850	**231**	463 - 1850	**231 – 463**	925 – 1850
1,1,1-trichloroethane	58 – 1850	negative	952 - 1850	negative	925 – 1850
1,2,3-trichloropropane	29 – 1850	negative	463 - 1850	**58 – 231**	463 – 925
1,2-dibromo-3-chloropropane	0.09 – 185	**46.3 – 92.5**	185	**2.9 - 185**	negative
tetrahalogenalkanes					
carbon tetrachloride	5.8 - 185	**92.5 - 185**	negative	negative	negative
haloketones					
1,1-dichloroacetone	0.09 - 185	**185**	negative	**5.8**	11.6 – 185
1,3-dichloroacetone	0.09 - 185	**0.19 – 0.38**	0.75 - 185	**0.38 – 0.75**	1.5 – 185
1,1,1-trichloroacetone	0.58 – 18.5	negative	negative	negative	negative
haloacetonitriles					
chloroacetonitrile	0.09 – 185	negative	negative	negative	1.5 – 185
dichloroacetonitrile	0.09 – 185	**11.6 – 23.1**	46.3 – 185	**5.8 – 23.1**	46.3 – 185
trichloroacetonitrile	0.09 – 185	**1.5**	2.9 – 185	**1.5**	2.9 – 185
bromochloroacetonitrile	0.09 – 185	negative	5.8 – 185	**0.75 – 1.5**	2.9 – 185
bromoacetonitrile	0.09 – 185	negative	5.8 – 185	negative	1.5 – 185
dibromoacetonitrile	0.09 – 185	negative	11.6 - 185	**0.75 – 1.5**	2.9 - 185
haloacetic acids					
chloroacetic acid	15 – 1850	negative	925 – 1850	negative	463 – 1850
dichloroacetic acid	15 – 1850	**1850**	negative	**231 – 1850**	negative
trichloroacetic acid	15 – 1850	negative	negative	negative	negative
bromoacetic acid	15 – 1850	negative	29 – 1850	negative	29 – 1850
dibromoacetic acid	15 – 1850	**58 – 231**	463 – 1850	negative	231 – 1850
tribromoacetic acid	15 – 1850	**231 – 463**	925 – 1850	**116 – 463**	925 – 1850
bromodichloroacetic acid	15 – 1850	**463**	925 – 1850	**925**	1850
dibromochloroacetic acid	15 – 1850	**116**	231 – 1850	**116 – 463**	925 – 1850
bromochloroacetic acid	15 – 1850	**116 - 925**	1850	**116 - 925**	1850
halonitroalkanes					
trichloronitromethane	0.18 - 185	**0.75 – 1.5**	2.9 - 185	**1.5 – 2.9**	5.8 - 185
haloaldehydes					
chloral hydrate	0.9 - 1850	negative	1850	**463 - 925**	1850

Investigation of swimming pool water extracts
More than 300 swimming pool water extracts were assessed by the umu-test with two different tester strains. At a concentration factor of 185 the GSTT1-tester strain showed a genotoxic effect in approx. 60% of the samples; the tester strain without GSTT1 only in 3%. A genotoxic effect was the more probable, the higher the concentration of AOX, total chlorine and/or THM in the original sample was. Statistically, it could be determined that 90% of the investigated extract samples showing a genotoxic effect in the umu test (GSTT1 tester strain) had concentrations of >300 µg/L AOX, >0.40 mg/L total chlorine and >20 µg/L THM (not concentrated).

4.2 Search for effect involved single substances

The correlation between concentrations of single substances measured in the extract and the observed effect in the umu test (induction rate) was determined by linear regression analyses. Table 2 shows single DBPs, which could be identified in swimming pool water extracts. THMs, HANs and chloral hydrate were found regularly (> 80% of samples). Substantially more rarely (<40% of samples), but in part relatively high concentrations 1,1,1-trichloroacetone (up to 24 µg/L), tetrachloroethene (up to 10 µg/L), trichloronitromethane (up to 7 µg/L) and 1.3-dichloroacetone (up to 6 µg/L) were observed. Methodically justified dihalogenalkanes could only measured in 3% of the samples in very small concentration (<4 µg/L).

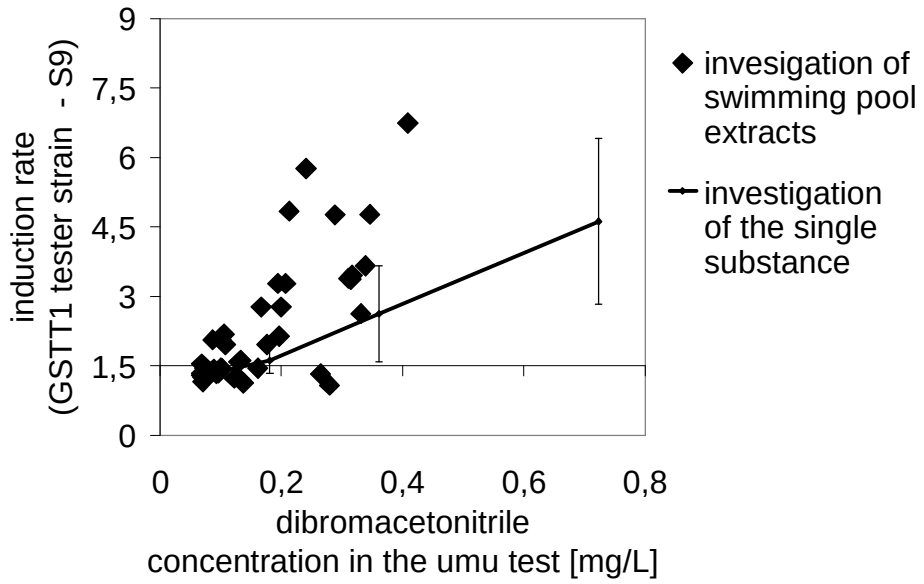
Table 3 shows linear regression analyses based on 72 data pairs. The data revealed a good correlation between the biological effect observed in the sample extract and its haloacetonitrile content. Single substances identified as being particularly effect-involved comprised dibromoacetonitrile and bromochloroacetonitrile. As pictures 1 and 2 show, effect concentrations of these two HANs were consistent with the effect concentration ranges, which were determined by testing the genotoxic potential of single substances.

Table 2: Concentration and occurrence frequency of 27 selected single DBPs in 72 swimming pool water extracts (liquid-liquid extraction with MTBE 10.000). n.m. = not measured.

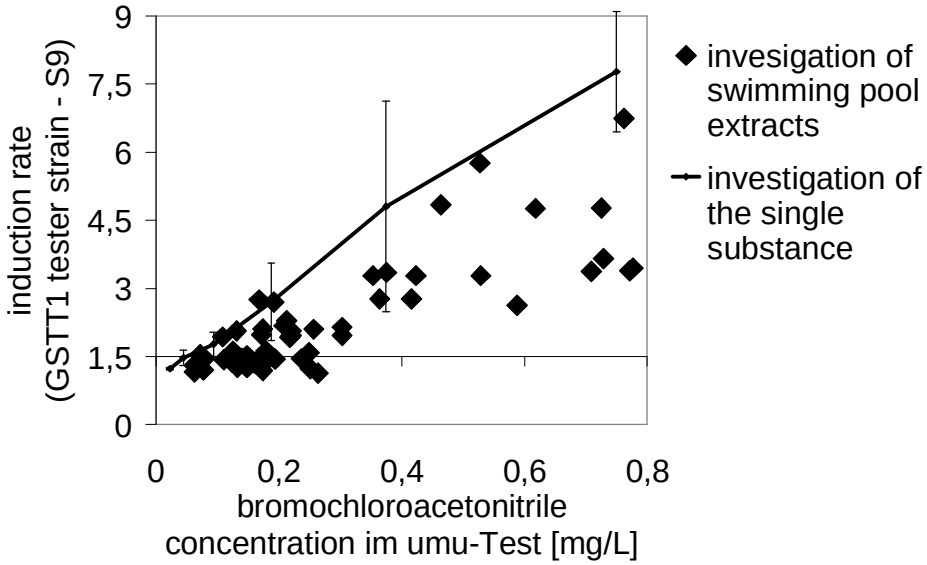
	frequency of occurrence [%]	extract concen-trations [µg/ 100 µL]	rate of recovery [%]	calculated concen-trations in swimming pool water [µg/L]	extract concen-trations in the umu test [mg/L]
haloalkanes					
chloroform	82	0 – 3	16	0 – 19	0 – 0.5
bromoform	39	0 – 2	44	0 – 9	0 – 0.3
bromodichloromthane	89	0 – 3	32	0 – 9	0 – 0.5
dibromochloromethane	90	0 - 4	40	0 - 10	0 – 0.8
1,1,1-trichloroethane	0	0	1	0	0
1,1,2-trichloroethane	0	0	38	0	0
1,2,3-trichloropropane	0	0	45	0	0
bromochloromethane	0	0	18	0	0
dichloromethane	0	0	n.m.	0	0
1,2-dichloroethane	1	0 – 0.5	13	0 – 4	0 – 0.1
1,2-dibromoethane	0	0	35	0	0
1-bromo-2-chloroethane	0	0	57	0	0
1,2-dibromo-3-chloropropane	3	0 - 1	46	0 - 2	0 – 0.2
haloalkenes					
trichloroethene	0	0	16	0	0
tetrachloroehene	1	0 - 2	20	0 - 10	0 – 0.3
halonitromethanes					
trichloronitromethane	15	0 - 2	29	0 - 7	0 – 0.3
haloacetonitriles					
chloroacetonitrile	0	0	7	0	0
dichloroacetonitrile	90	0 – 6	30	0 - 20	0 - 11
trichloroacetonitrile	1	0 – 2	13	0 – 15	0 – 0.4
bromoacetonitrile	3	0 – 1	8	0 – 13	0 – 0.1
dibromoacetonitrile	85	0 – 2	35	0 - 6	0 – 0.4
bromochloroacetonitrile	90	0 - 4	33	0 - 12	0 – 0.8
haloketones					
1,1-dichloroacetone	0	0	18	0	0
1,3-dichloroacetone	3	0 – 0.5	8	0 - 6	0 – 0.1
1,1,1-trichloroacetone	38	0 - 5	21	0 - 24	0 – 0.9
haloamides					
2,2-dichloroacetamide	17	0 - 1	n.m.	?	0 – 0.3
halogenated aldehydes					
chloral hydrate	100	1.5 - 450	n.m.	?	0.3 - 84

Table 3: Correlation of extract constituents with the observed induction rate (concentration factor 185) in the umu test (GSTT1-tester strain) based on a linear regression analysis using 72 data pairs in each case. Given are the maximum extract concentration and the effect threshold noticed in the umu test. The effect threshold marks the concentration range between the lowest-observed-adverse-effect level (LOAEL) and the no-observed-effect level (NOEL), determined by single substance testing.

	maximal concentration in extracts (185:1) examined in the umu test in mg/L	**effect threshold in mg/L**	**correlation of substance concentrations in the extract with induction rates of the extracts (185: 1) in the GSTT1 tester strain (- S9) (linear regression analysis)**
DBP sum parameters			
sum of all detected DBPs			$y = -0.002x + 2.66$. $r^2 < 0.1$
sum of haloacetonitriles			**$y = 0.40x + 0.32$. $r^2 = 0.5$**
sum of haloalkanes			$y = 0.20x + 1.50$. $r^2 = 0.5$
sum of haloketones			$y = 0.72x + 2.44$. $r^2 < 0.1$
DBP single substances			
bromochloroacetonitrile	**0.8**	**0.38 – 0.75**	**$y = 0.83x + 0.82$. $r^2 = 0.5$**
dibromoacetonitrile	**0.4**	**0.38 – 0.75**	**$y = 1.99x + 0.48$. $r^2 = 0.5$**
dichloroacetonitrile	1.1	2.9 – 5.8	$y = 0.60x + 1.20$. $r^2 = 0.2$
trichloroacetonitrile	0.4	0.75 – 1.5	$y = 0.40x + 2.29$. $r^2 < 0.1$
1,3-dichloroacetone	0.1	0.19 – 0.38	$y = 0.001x + 0.01$. $r^2 < 0.1$
trichloroacetone	0.9	negative up to 18.0	$y = 0.01x + 0.01$. $r^2 < 0.1$
trichloronitromethane	0.3	0.75 – 1.5	$y = -0.28x + 2.28$. $r^2 < 0.1$
1,2-dibromo-3-chloropropane	0.1	0.91 – 1.81	$y = -0.003x + 0.01$. $r^2 < 0.1$
1,2-dichloroethane	0.2	105 – 2.9	$y = 3.56x + 2.27$. $r^2 = < 0.1$
dibromochloromethane	0.8	116 - 231	$y = 0.36x + 0.46$. $r^2 = 0.2$
bromodichloromethane	0.5	116 - 231	$y = 0.25x + 0.79$. $r^2 = 0.1$
bromoforme	0.3	116 - 231	$y = 0.16x + 0.05$. $r^2 = 0.1$
chloral hydrate	84	231 - 463	$y = -0.001x + 2.65$. $r^2 < 0.1$
chloroforme	0.5	negative	$y = 0.01x + 1.41$. $r^2 = 0.1$
tetrachloroethene	0.3	negative	$y = 0.001x + 0.003$. $r^2 < 0.1$



Picture 1: Dependence of the bromochloroacetonitrile concentration of the extract on the induction rate of the extract (185:1) in the GSTT1- tester strain without S9 (black points). For comparison the dose effect relation of the single substance in the umu test is shown (black line). Induction rates >1.5 indicate a genotoxic effect in the umu test.

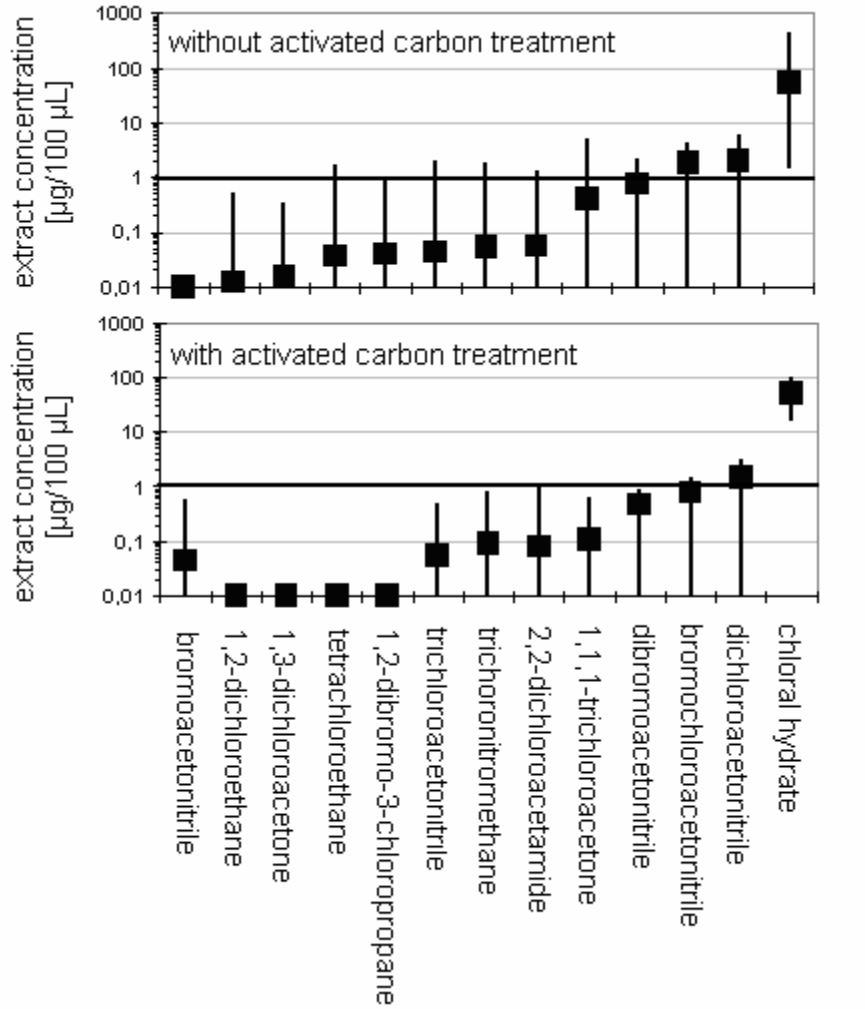


Picture 2: Dependence of the dibromoacetonitrile concentration of the extract on the induction rate of the extract (185: 1) in the GSTT1- tester strain without S9 (black points). For comparison the dose effect relation of the single substance in the umu test is shown (black line). Induction rates >1.5 indicate a genotoxic effect in the umu test.

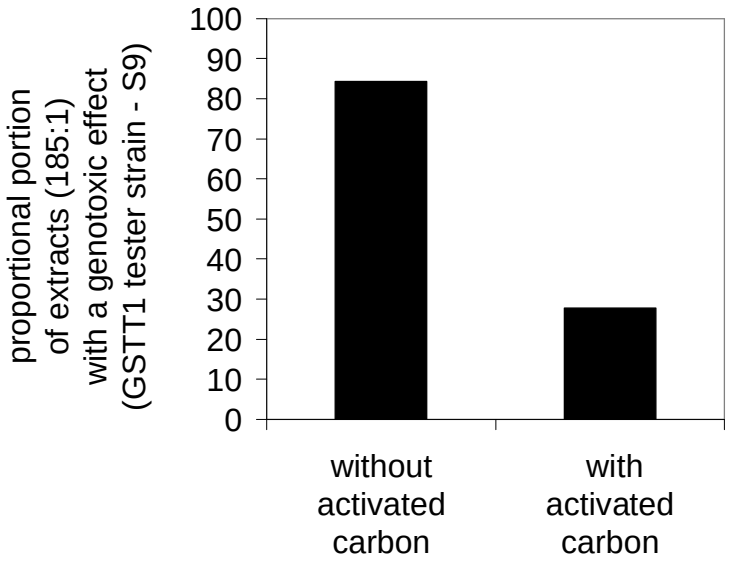
4.3 Impact of water treatment on HAN concentration

Swimming pool water extracts from an indoor pool, which was treated with powdered activated carbon showed more rarely a genotoxic effect (GSTT1-tester strain) than extracts from a similar pool without any activated carbon treatment (picture 4). Analytics by GC/MS

of the pool water extracts confirmed substantially smaller DBP concentrations in the basin water, which was treated with powdered activated carbon (picture 3). Most interestingly, also the concentrations of bromochloroacetonitrile and dibromoacetonitrile, which are probably effect-responsible, differed. Their concentrations reached 1 µg/L and more without activated carbon treatment, while they were under 1µg/L with activated carbon. Results from this example indicate that activated carbon filtration might be a good treatment option for haloacetonitrile removal from pool water, because the single substances showed high sorption tendencies towards activated carbon.



Picture 3: Comparison of DBP concentrations in swimming pool water extracts from two non swimmer basins, which are treated with or without powdered activated carbon. The average value from 15 samples is shown in each case as well as the minimum and the maximum concentration.



Picture 4: Proportional portion of swimming pool water extracts with a genotoxic effect in the umu test (GSTT1 tester strain without S9). In each case 15 samples from two non swimmer basins have been examined, which are treated with or without powdered activated carbon.

4.4 Correlation of HANs and THMs in swimming pool water

The parameter THM in the swimming pool water could be correlated with the concentration of the haloalkanes as well as the HANs in the concentrated samples. This result indicates that HAN concentrations could presumably be controlled by existing and future THM regulations.

5. Discussion

5.1 Occurrence of HANs in swimming pool water

Beside trihalomethanes (THMs), haloacetic acids (HAA), haloketones, chloral hydrate and chloropicrin, HANs represent another dominating DBP group in swimming pool water. Since in most baths chloride concentrations are higher than bromide concentrations, also chlorinated compounds quantitatively dominate within the individual DNP groups. HANs form via the reaction of organic nitrogen compounds with chlorine. Increasing temperatures and decreasing pH values favour the formation of HANs (5). Due to elevated temperatures in swimming pools and the continuous input of nitrogenous compounds by the bathers (via urine or sweat) the amount of HANs in swimming pool water could be much higher than in drinking water.

5.2 Toxicological properties – what do we know?

HANs are well-known as direct acting toxicants, which can react with haemoglobin, DNA and other macromolecules. They have been tested in various in-vitro cancer and genotoxicity screening assays. The heterogeneous rank order of toxicity of individual HAN compounds is related to the different test systems used. HANs turned out to be genotoxic *in-vitro* (SOS Chromotest, SCE test, Comet Assay) and to initiate skin and lung tumours in mice (6, 7, 8). Dibromoacetonitrile, however, was non-mutagenic in the Ames tester strain TA100 (9). The strong cytotoxic potential of some HANs is probably based on the formation of cyanide (10). Additionally, HANs are known to cause oxidative stress and damage in cells via

metabolically formed reactive oxygen species (ROS) resulting in an increase of both apoptosis and necrosis. Furthermore, certain HANs can inhibit detoxifying enzymes (11).

5.3 Regulations

HANs are a class of DBPs that is not regulated in swimming pool water. At present, the carcinogenic potential of HANs for humans cannot be fully evaluated due to an insufficient database (no appropriate human data, mostly no chronic animal study). The WHO Guidelines for drinking-water quality classified HANs in 2004 as „possibly carcinogen to humans“(12). The stated guideline value for dibromoacetonitrile is 0.07 mg/litre based on a subchronic study in the rat. For dichloroacetonitrile, the WHO reports only a provisional guideline value of 0.02 mg/litre due to limitations of the toxicological database. For bromochloroacetonitrile, the insufficient database even precluded derivation of a health-based guideline value. HANs were recommended as priority candidates for future carcinogenicity studies in a selection study of the US EPA (2002), in order to use the results for regulations of drinking water (13). Dibromoacetonitrile, however, was only recently evaluated for chronic toxicity and carcinogenicity. In a two-year study with rodents, reported in 2008 (14), a clear evidence of carcinogenic activity of dibromoacetonitrile in male rats, some evidence of carcinogenic activity in female rats, and clear evidence of carcinogenic activity in male and female B6C3F1 mice was shown. However, drinking water limit values cannot be transferred directly to swimming pool water, because of different exposition conditions and pathways. Drinking water guidelines assume an oral lifelong consumption of 2 litres per day. Ingestion of swimming pool water is considerably lower, but skin absorption and inhalation of DBPs can be proportionally relevant. In order to define health-based guideline values for HANs in swimming pool water, risk evaluation needs to be improved, which includes toxicological data, toxicokinetics and the knowledge on various exposition routes (dermal vs. oral). Regardless of the current gaps in regulation; the fact that the HAN concentration in swimming pool water can be controlled by state-of-the-art methods already frequently applied in practice (activated carbon adsorption) allows, according to the precautionary principle, for an exclusion of a possible risk via adequate water treatment.

6. Open questions

A recent epidemiological study (15) indicates that the risk of bladder cancer associated with drinking water might be related mainly to three factors: THM levels, showering/bathing/swimming and genotypus of the individuum (having the GSTT1 gene). Based on the fact that HANs are a major group of DBPs in swimming pool water, that HAN concentrations seem to correlate with THM concentrations and that some HANs are probably activated to genotoxicants via conjugation by GSTT1, more emphasis should be put on the relevance of HAN toxicity in swimming pool water in future.

6.1 Exists a health risk by HANs when bathing in the swimming pool?

The present study revealed that dibromoacetonitrile and bromochloroacetonitrile are activated to genotoxicants by the product of the glutathione S-transferase- (GSTT1) gene in *Salmonella* TA1535/NM5004 being transfected with this gene. To evaluate this phenomenon in humans, in vitro tests with mammalian cells, in vivo toxicological studies and long-term tests for chronic toxicity and carcinogenicity are needed. Furthermore, understanding of the toxicity mechanism will allow for a better understanding of the relationships between detoxifying and

activating reactions in human target cells and of organ-specific effects. Even if a compound can be classified as genotoxic or cancerogenic, it is not clear yet whether and on which exposition conditions it can get into the body. Physiologically based pharmacokinetic (PBPK) models for humans are needed to quantify total exposure to relevant concentration levels from multiple exposure routes and to predict concentrations of target tissues. Finally, risk assessment studies have to evaluate the relationship between exposure, internal dose, and toxicity.

6.2 Can HANs be taken up by multiple exposition paths from swimming pool water?

Recent findings highlight the emerging importance of dermal or inhalative exposure pathways from chlorinated drinking water or swimming pool water. It was shown, for instance, that extensive usage of hot water during showering, bathing and manual dishwashing correlated with an increase of THM concentrations in blood and exhalation (16). Furthermore, also inhalation and dermal absorption of HANs during showering can make up about 30 percent of the dose being ingested by consumption of chlorinated drinking water (17). As a consequence, these aspects need to be considered also for the evaluation of HAN risks from swimming pool water. Further research is needed to determine whether acute and repeated frequent exposure to relevant HAN concentrations via swimming pool water have public health implications.

6.3 Are certain human subpopulations more susceptible?

Because the GSTT1-gene is polymorphic in humans, with 20–25% of Caucasians and 50% of Asians having a homozygous deletion of this gene (null genotype) (18, 19), the results in *Salmonella* might indicate that people carrying the GSTT1-gene are possibly more susceptible to the genotoxic effects of the brominated dihalogenated HANs than those missing the gene.

Acknowledgement

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