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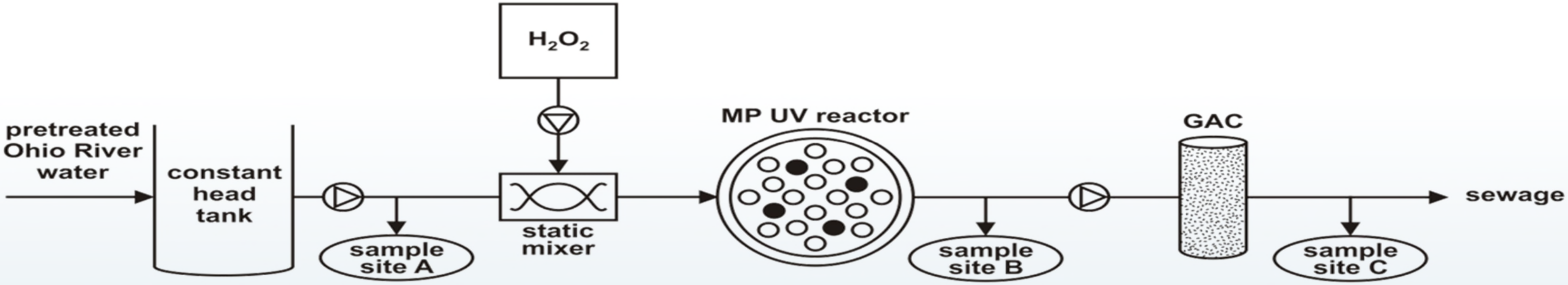
Application of effect-directed analysis to identify mutagenic nitrogenous disinfection by-products of advanced oxidation drinking water treatment

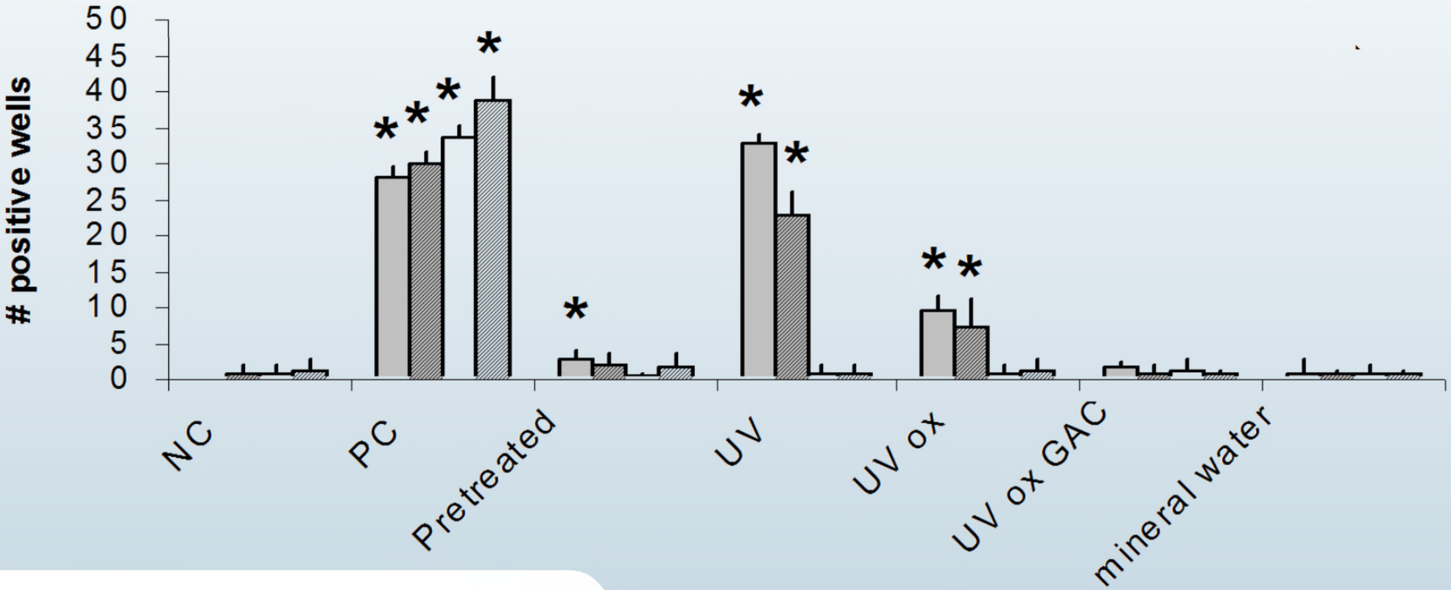
KWR Watercycle Research Institute Kirsten Baken, Dennis Vughs, Annemieke Kolkman
PWN Bram Martijn

Chemical analysis and bioassays in the drinking water production train

- **Surface water is treated to produce drinking water**
  - Disinfection (*chlorination, ozonation, UV radiation*)
  - Removal of micropollutants (*adsorption, membranes, advanced oxidation*)
  - Advanced oxidation processes (AOP) involving UV and ozone effectively remove persistent pathogens and polar chemical contaminants (e.g. pharmaceuticals, pesticides)
- **AOP may generate mixtures of disinfection by-products (DBPs)**
  - More than 600 (*chlorinated, iodinated, brominated, nitrogenous*) DBPs have been identified but many more are formed
  - Chemical identity and toxicity are often not known
- **Ames fluctuation test detects the presence of unknown, potentially mutagenic N-DBPs**
  - Genotoxicity has been observed in water treated by medium pressure (MP) UV/H_2O_2

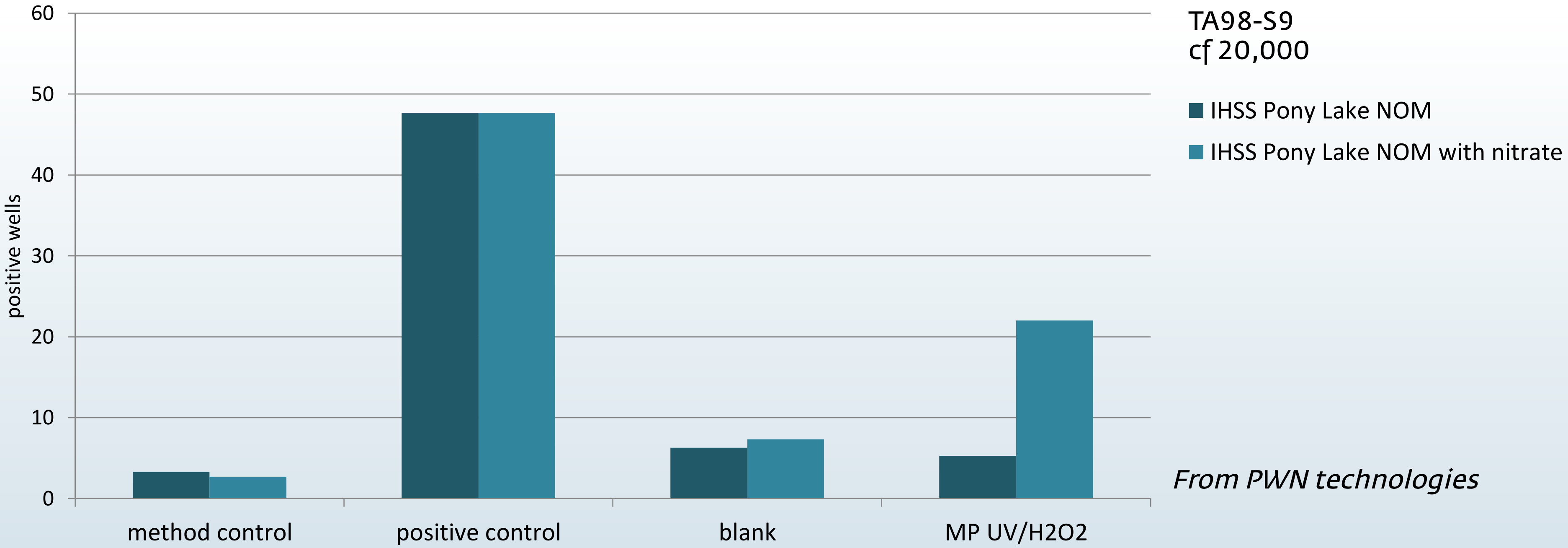
Mutagenic byproducts of MP UV treatment





Heringa et al., 2011

Role of Natural Organic Matter (NOM) and nitrate



From PWN technologies

Nitrate photolysis by MP UV irradiation in the presence of NOM was found to be the key parameter in the manifestation of an Ames test response; nitro radicals as (reactive) intermediates?

Martijn et al., 2014

Identification of disinfection by-products

Aim of the study

- Provide information on the chemical reactions and process conditions involved in their formation
- Study of the behavior and fate during drinking water treatment
- Perform human health risk assessment

Environmental Science & Technology
Article
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Tracing Nitrogenous Disinfection Byproducts after Medium Pressure UV Water Treatment by Stable Isotope Labeling and High Resolution Mass Spectrometry

Annemieke Kolkman,*[,†] Bram J. Martijn,[‡] Dennis Vughs,[†] Kirsten A. Baken,[†] and Annemarie P. van Wezel[†,§]

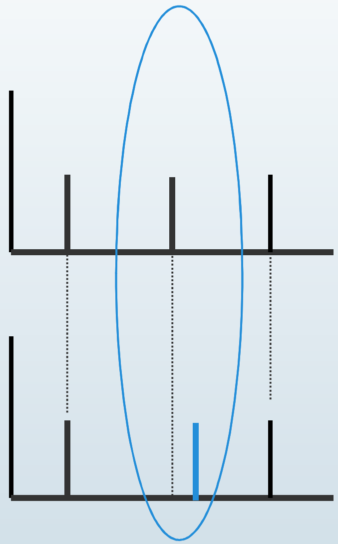
Development of an innovative tool for the detection of N-DBPs of MP UV treatment

Nitrogen labeling principle

NOM + nitrate (NO_3^-) + MP UV → nitrogen containing by-products

NOM + $^{14}NO_3^-$ + MP UV → nitrogen containing by-products

NOM + $^{15}NO_3^-$ + MP UV → nitrogen containing by-products



Isotope tagging in the mass spectrometer

Kolkman et al., 2015

Results

84 N-DBPs detected

Negative mode

- 78 detected compounds
- 54 different chemical formulas
- 14 compounds with 2x ^{15}N label
- Total concentration = 1234 ng/l (bentazon-d6)

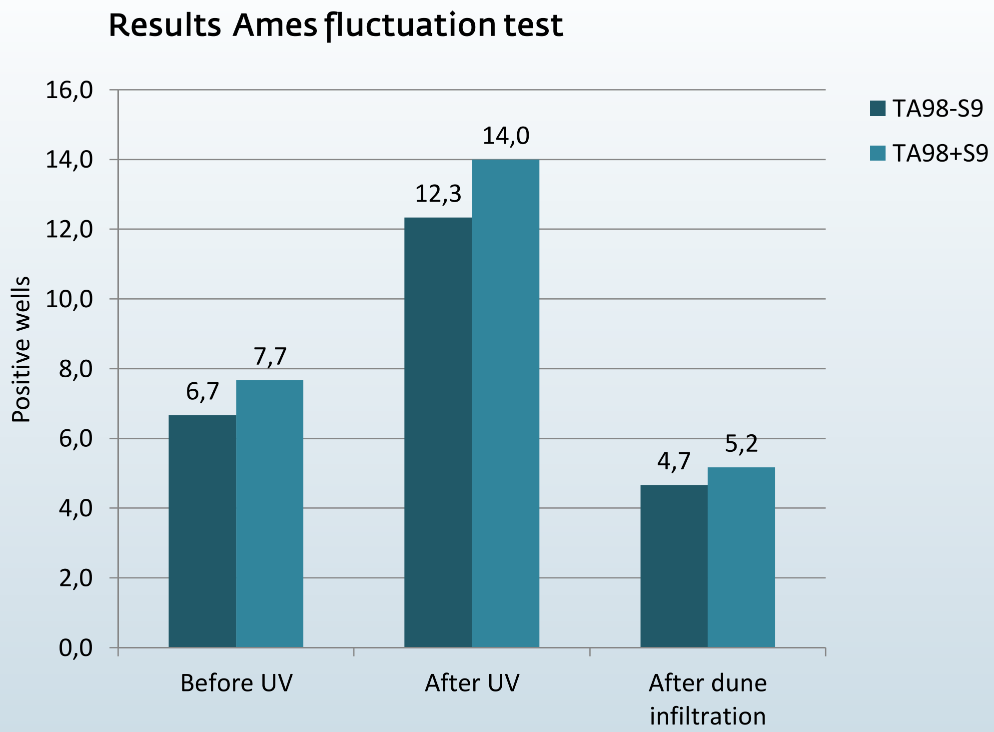
Positive mode

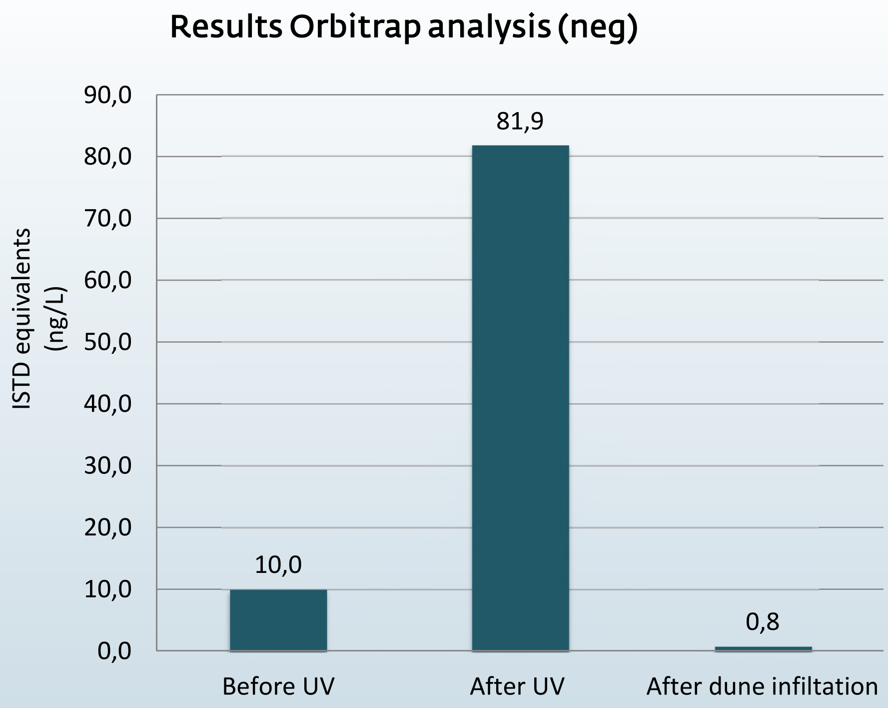
- 16 detected compounds
- 6 different chemical formulas
- 0 compounds with 2x ^{15}N label
- Total concentration = 69 ng/l (atrazin-d5)
- 6 compounds detected only in positive mode

Kolkman et al., 2015

Full scale water treatment

Results bioassays versus chemical analysis





Kolkman et al., 2015

Results

Genotoxic potential of identified N-DBPs

Compound	CAS nr	Formula	Genotoxic potential (based on measured data* and/or QSAR analysis)
4-nitrophenol	100-02-7	$C_6H_5NO_3$	Overall evidence points to absence of mutagenicity in Ames test; insufficient data to assess other genotoxicity and carcinogenic potential.*
4-nitrocatechol	3316-09-4	$C_6H_5NO_4$	Probably not mutagenic in Ames test; insufficient data to assess other genotoxicity and carcinogenic potential.
4-nitro-1,3-benzenediol	3163-07-3	$C_6H_5NO_4$	Structure suggests genotoxic potential.
2-nitrohydroquinone	16090-33-8	$C_6H_5NO_4$	Structure suggests genotoxic potential.
2-hydroxy-5-nitrobenzoic acid	96-97-9	$C_7H_5NO_5$	Structure suggests genotoxic potential but no mutagenicity.
4-hydroxy-3-nitrobenzoic acid	616-82-0	$C_7H_5NO_5$	Structure suggests genotoxic potential.
2-hydroxy-3-nitrobenzoic acid	85-38-1	$C_7H_5NO_5$	Structure suggests genotoxic potential.
2,4-dinitrophenol	51-28-5	$C_6H_4N_2O_5$	Weight-of-evidence indicates no mutagenicity and genotoxicity, but clastogenicity and carcinogenicity cannot be excluded.*
5-nitrovanillin	6635-20-7	$C_8H_7NO_5$	Structure suggests genotoxic potential but no mutagenicity.
4-nitrobenzenesulfonic acid	138-42-1	$C_6H_5NO_5S$	Mutagenicity and genotoxicity are not expected.*
4-nitrophthalic acid	610-27-5	$C_8H_5NO_6$	Structure suggests genotoxic potential.
2-methoxy-4,6-dinitrophenol	4097-63-6	$C_7H_6N_2O_6$	Potentially mutagenic in Ames test; insufficient data to assess other genotoxicity and carcinogenic potential.
3,5-dinitrosalicylic acid	609-99-4	$C_7H_4N_2O_7$	Structure suggests genotoxic potential.
dinoterb	1420-07-1	$C_{10}H_{12}O_5N_2$	Structure suggests genotoxic potential.

Kolkman et al., 2015

Effect directed analysis approach

Aim of the study

- Genotoxic potential of the identified N-DBPs does not explain the observed Ames response
- Application of effect directed analysis to identify mutagenic nitrogenous disinfection byproducts

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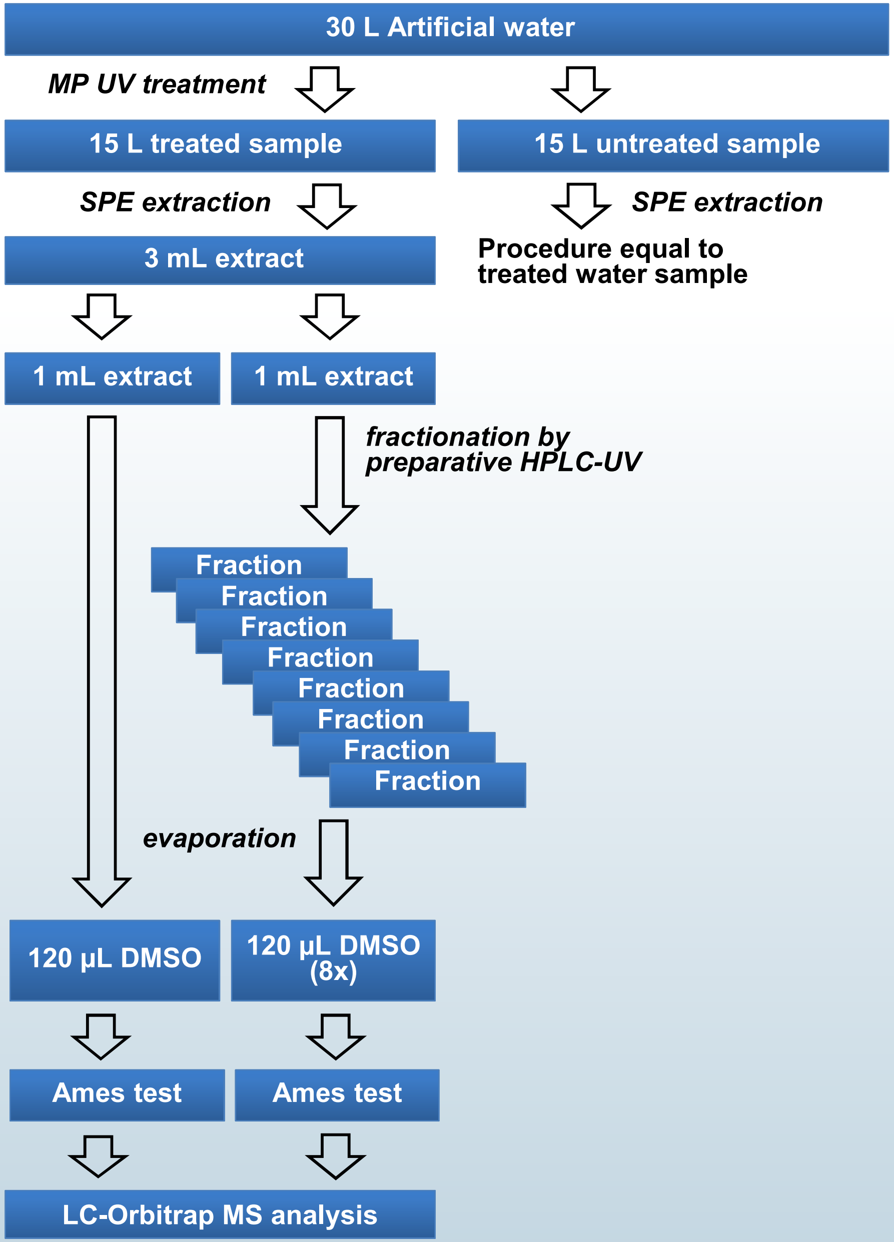
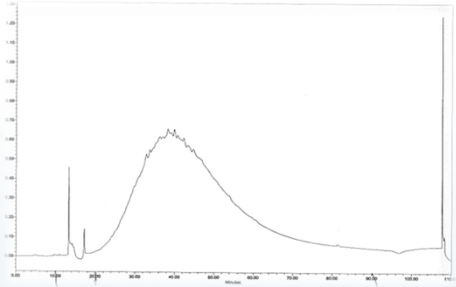
EFFECT-RELATED EVALUATION OF ANTHROPOGENIC TRACE SUBSTANCES, -CONCEPTS FOR GENOTOXICITY, NEUROTOXICITY AND, ENDOCRINE EFFECTS

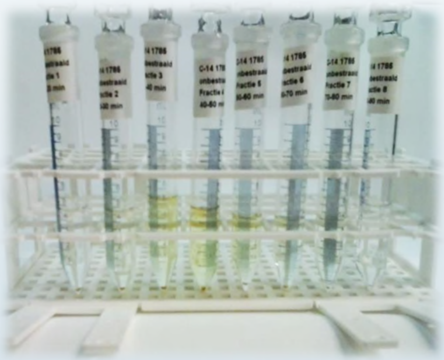
Application of effect-directed analysis to identify mutagenic nitrogenous disinfection by-products of advanced oxidation drinking water treatment

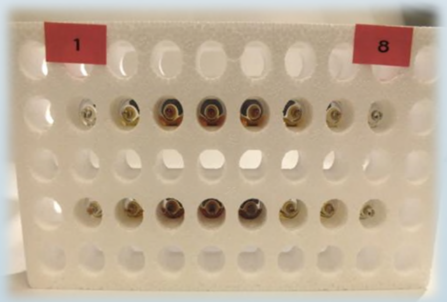
D. Vughs[1] · K. A. Baken[1] · A. Kolkman[1] · A. J. Martijn[2] · P. de Voogt[1,3]

Experimental design





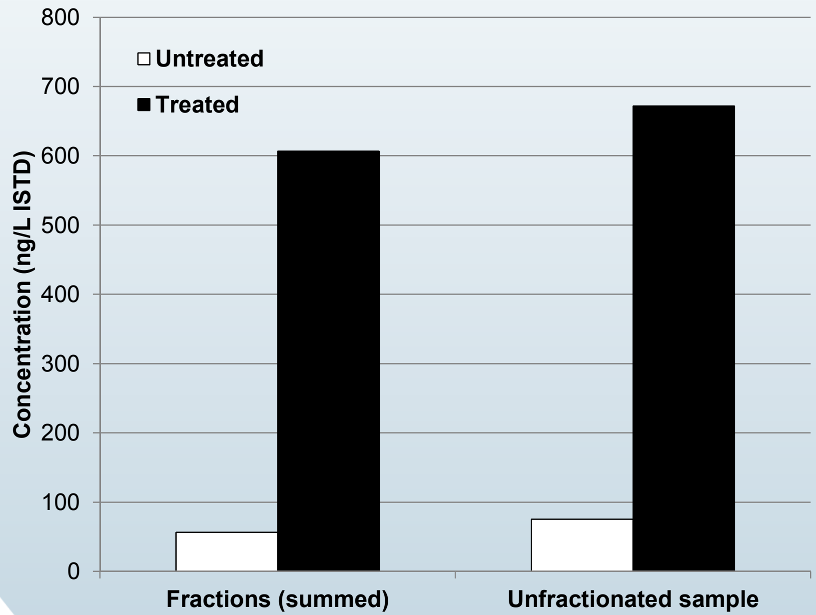




Vughs et al., 2015

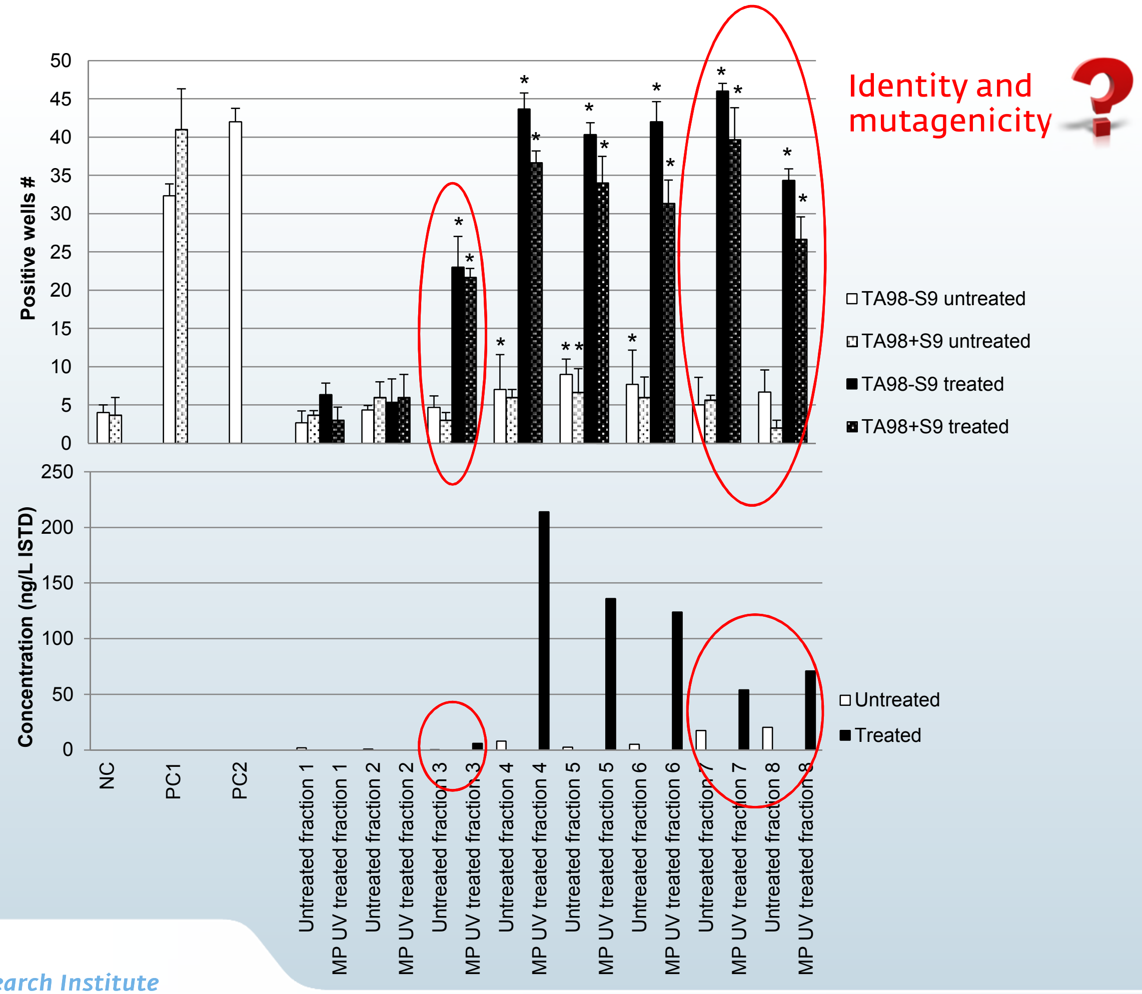
Fractionation and concentration of water extracts

- The total concentration of byproducts detected in the fractionated samples was in agreement with the total concentration detected in the unfractionated samples
- The majority of the N-DBPs were shown to be predominantly present in one of the fractions



Vughs et al., 2015

N-DBPs in fractionated water extracts



Vughs et al., 2015

Top 5 of N-DBPs per fraction

Mass (m/z)	Conc. (ng/L)	Formula	Compound
Fraction 3			
400.1262 (1)	1.9		
386.1096 (1)	1.3		
154.0148 (1)	0.8	$C_6H_5O_4N$	4-nitrocatechol
210.0048 (1)	0.7	$C_8H_5O_6N$	4-nitrophthalic acid
442.1365 (2)	0.4		
Fraction 4			
182.0098 (2)	42.2	$C_7H_5O_5N$	4-hydroxy-3-nitrobenzoic acid
138.0198	29.2	$C_6H_5O_3N$	4-nitrophenol
154.0148 (1)	26.2	$C_6H_5O_4N$	4-nitrocatechol
400.1262 (2)	10.6		
408.1308 (2)	10.0		
Fraction 5			
316.1413 (1)	34.9	$C_{14}H_{23}O_7N$	
208.0255	7.9	$C_9H_7O_5N$	
452.1203 (2)	7.7		
225.9994 (2)	7.4	$C_8H_5O_7N$	
213.0154	6.9	$C_7H_6O_6N_2$	2-methoxy-4,6-dinitrophenol

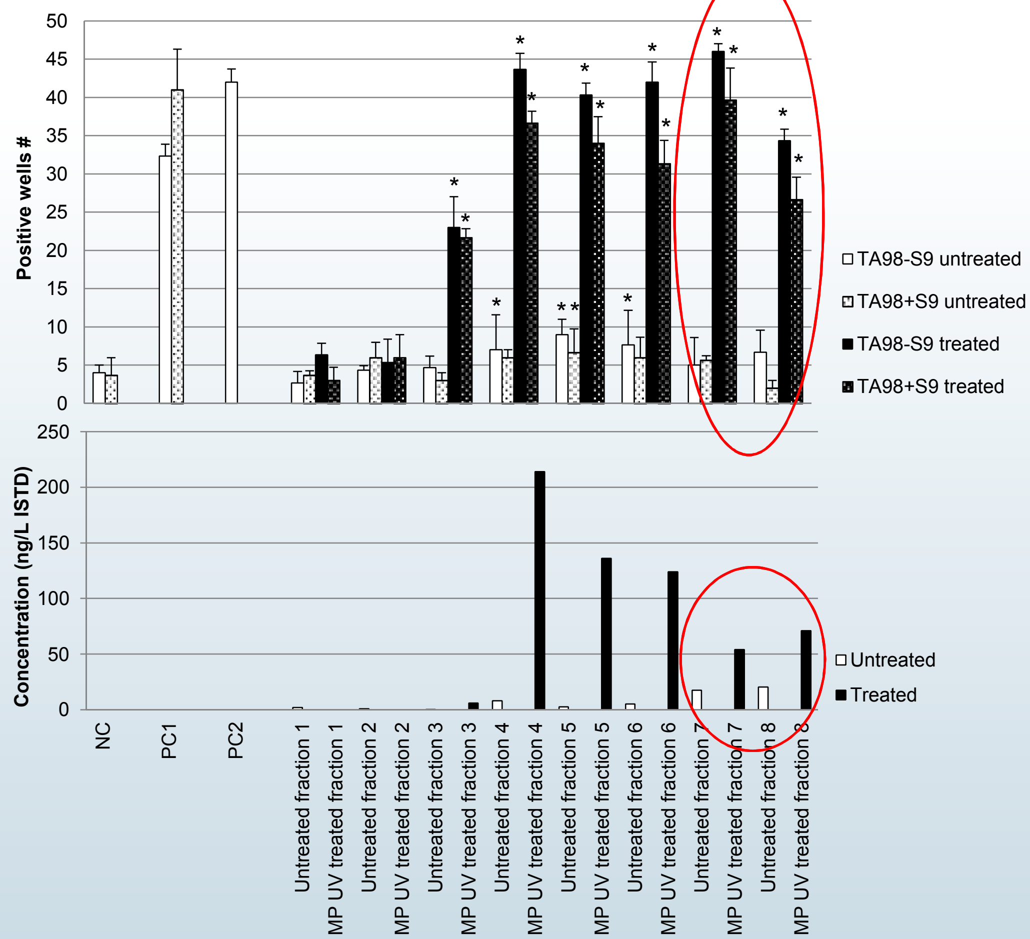
Mass (m/z)	Conc. (ng/L)	Formula	Compound
Fraction 6			
213.0154	38.5	$C_7H_6O_6N_2$	2-methoxy-4,6-dinitrophenol
316.1413 (3)	11.7	$C_{14}H_{23}O_7N$	
238.0726	9.0	$C_{11}H_{13}O_5N$	
270.0755 (1)	9.0		
316.1413 (1)	8.3	$C_{14}H_{23}O_7N$	
Fraction 7			
212.0204	23.9	$C_8H_7O_6N$	Structural isomer of 5-hydroxy-4-methoxy-2-nitrobenzoic acid
266.1037	8.4	$C_{13}H_{17}O_5N$	
239.0677	8.0	$C_{10}H_{12}O_5N_2$	dinoterb
153.0073	5.3		
226.9948	1.8	$C_7H_4O_7N_2$	3,5-dinitrosalicylic acid
Fraction 8			
182.0098 (3)	56.2	$C_7H_5O_5N$	2-hydroxy-5-nitrobenzoic acid
226.9948	5.5	$C_7H_4O_7N_2$	3,5-dinitrosalicylic acid
196.0258 (3)	3.9		
372.1491	2.1		
239.0677	0.6	$C_{10}H_{12}O_5N_2$	dinoterb

Based on (predicted) genotoxic potential 4-nitrophthalic acid, 4-hydroxy-3-nitrobenzoic acid, 2-methoxy-4,6-dinitrophenol, dinoterb and 3,5-dinitrosalicylic acid may have contributed to the observed mutagenicity.

Vughs et al., 2015

Which N-DBPs explain mutagenicity in fraction 7 and 8?

Mass (m/z)	RT (min)	Mode	fraction	Conc. (ng/L)	Formula	ID
340.1388 (1)	27.80	pos	7	0.3	$C_{16}H_{21}O_7N$	
340.1388 (2)	28.16	pos	7	1.3	$C_{16}H_{21}O_7N$	
340.1388 (3)	28.90	pos	8	0.3	$C_{16}H_{21}O_7N$	
239.0677	26.78	neg	7	8.0	$C_{10}H_{12}O_5N_2$	Dinoterb
372.1491	24.99	neg	8	2.1	?	



Vughs et al., 2015

Conclusions

- **Nitrogen labeling is a new innovative approach for the detection of nitrogen containing by-products**
- **By applying a fractionation method to MP UV treated water samples, the presence of N-DBPs and mutagenicity in the Ames test were shown to be correlated**
- **A selection of byproducts that are likely to contribute to the mutagenic response were identified**

- Outlook
  - Testing of (mixtures of) the N-DBPs in the Ames fluctuation tests
  - Identification and quantification of additional by-products
  - Relevance for full-scale treatment and varying process conditions (*water composition, AOP conditions*)
  - Refinement of methodology (*number of fractions, bioassay panel*)

Thanks for your attention!

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