Tracing genotoxic disinfection by-products after medium pressure UV water treatment using nitrogen labeling, mass spectrometry and effect directed analysis

Dennis Vughs, Annemieke Kolkman, Kirsten Baken, Bram Martijn, Annemarie van Wezel and Pim de Voogt

Water treatment
formation by-products

- **Water treatment of surface water**
  - Disinfection (chlorination, ozonation, UV radiation)
  - Removal of micro pollutants (adsorption/GAC, RO membrane, advanced oxidation (UV, ozone))

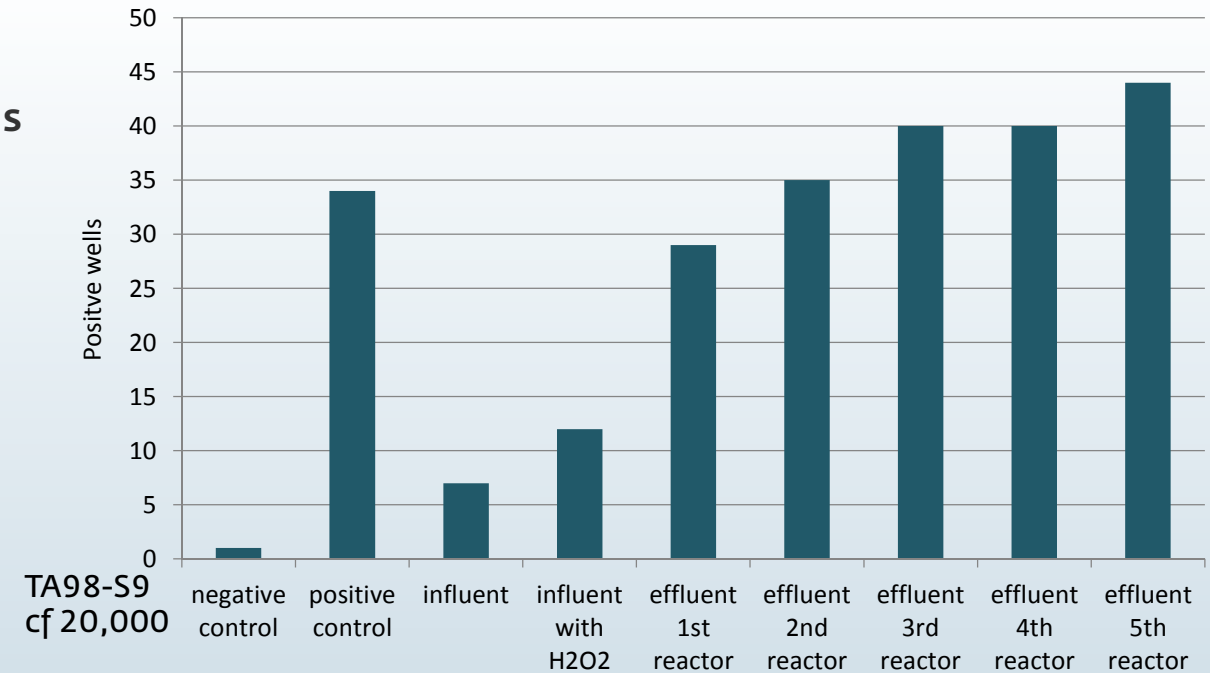
- **Water treatment may cause by-products**
  - THM's, HAA's (chlorination)
  - Bromate (ozone)
  - Nitrite (MP UV)

MP UV water treatment

Ames test response after MP UV/H_2O_2 treatment at wtp Heemskerk

- **Increased AMES test response observed**
- **Is an indication of genotoxic compounds**
- **What is the cause?**

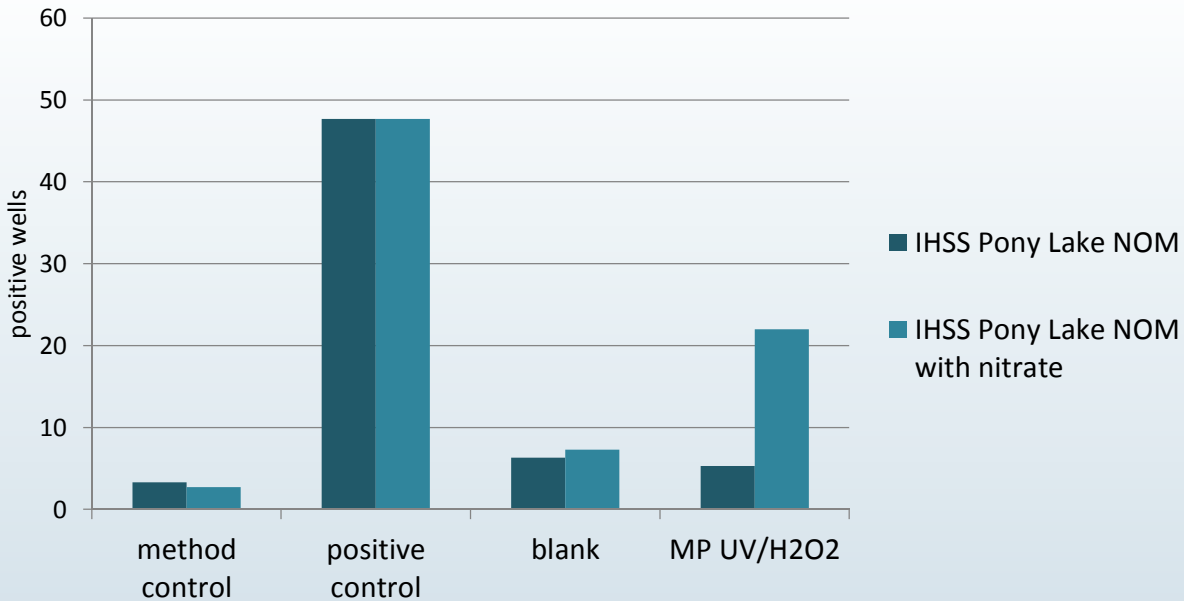
- probably caused by the formation of by-products



Source: PWN technologies

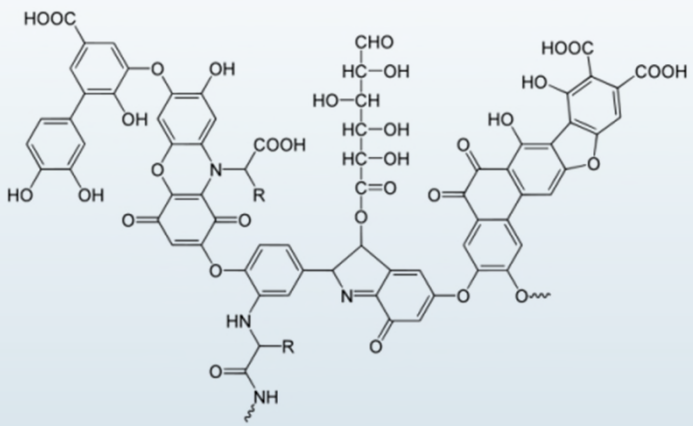
Introduction

Ames response after MP UV/H_2O_2 treatment in artificial water



TA98-S9
cf 20,000

Example of humic acid



Source: PWN technologies

Introduction

MP UV treatment and Ames test

- **MP UV involves nitrate photolysis -> nitro radicals are formed**
- **May form nitro(so) organic compounds when both nitrate and NOM are present**
- **Effect measured**
  - no compound(s) identified
  - no concentration established
- **The identification is essential for risk assessment**
- **Development of a tool for the detection of by-products formed by MP UV treatment**

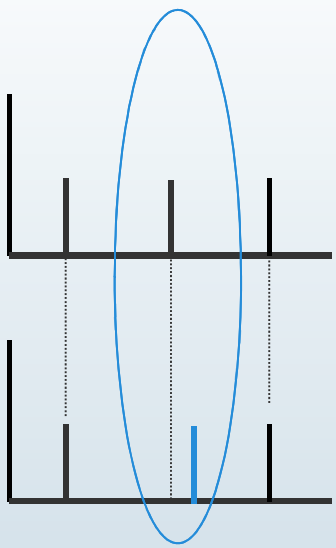
Introduction

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

Δ m/z = 0.99704

Kolkman et al, Environ. Sci. Technol. 2015, 49, 4458.4465

Experimental design

Overview

- Sample preparation → artificial water (ultrapure water + Pony Lake NOM + nitrate)

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- UV irradiation → collimated beam MP UV irradiation

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- Sample pretreatment → solid phase extraction

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- Analysis → LC-Orbitrap-MS

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- Data processing → differential analysis

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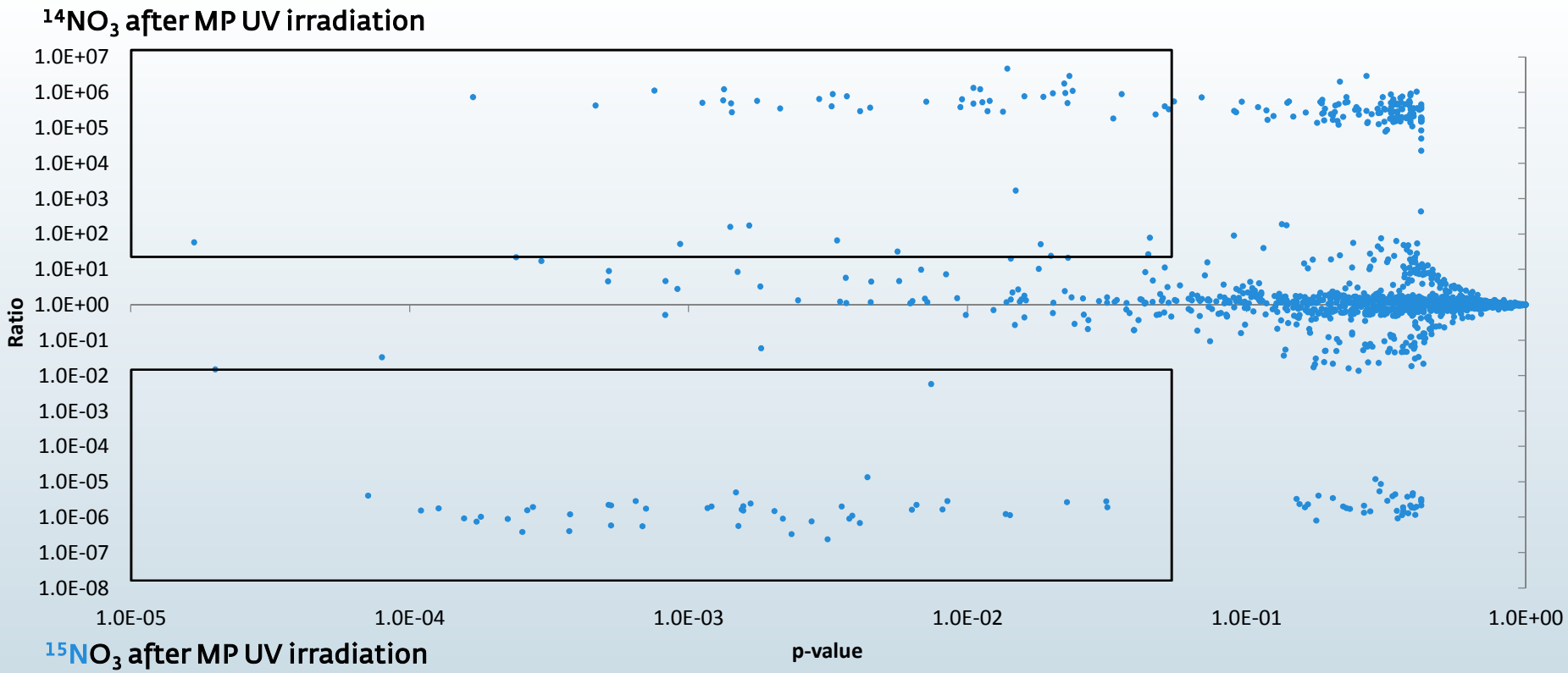
- Identification by-products

Data processing

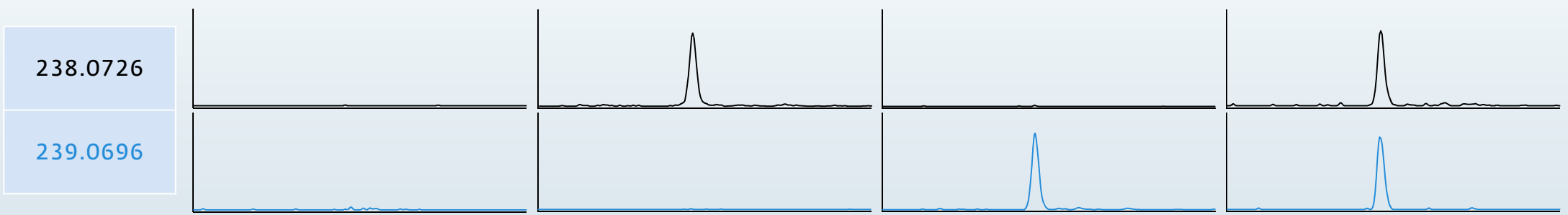
Volcano plot $^{14}NO_3$ vs $^{15}NO_3$ after MP UV (neg)



Results

Chromatograms (EIC) unknown compound m/z 238.0726

Sample	1	2	3	4
Nitrate	$^{14}NO_3^-$	$^{14}NO_3^-$	$^{15}NO_3^-$	$^{14}NO_3^-/^{15}NO_3^-$ (1:1)
MP UV	-	+	+	+



^{14}N = 14.00307 m/z
^{15}N = 15.00011 m/z
Difference = 0.99704 m/z

Kolkman et al, Environ. Sci. Technol. 2015, 49, 4458.4465

Results

Overview

Negative analysis

- 78 detected compounds
- 54 different chemical formulas
- 14 compounds with 2x ^{15}N label
- Total concentration = 1234 ng/L (ISTD eq.)

Positive analysis

- 16 detected compounds
- 6 different chemical formulas
- Total concentration = 69 ng/L (ISTD eq.)
- 6 compounds detected only in positive mode

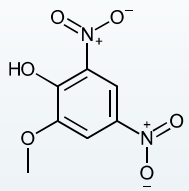
Kolkman et al, Environ. Sci. Technol. 2015, 49, 4458.4465

Results

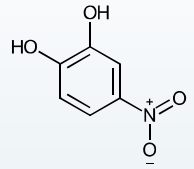
Identified N-DBPs

Compound	CAS nr	Formula
4-nitrophenol	100-02-7	$C_6H_5NO_3$
4-nitrocatechol	3316-09-4	$C_6H_5NO_4$
4-nitro-1,3-benzenediol	3163-07-3	$C_6H_5NO_4$
2-nitrohydroquinone	16090-33-8	$C_6H_5NO_4$
2-hydroxy-5-nitrobenzoic acid	96-97-9	$C_7H_5NO_5$
4-hydroxy-3-nitrobenzoic acid	616-82-0	$C_7H_5NO_5$
2-hydroxy-3-nitrobenzoic acid	85-38-1	$C_7H_5NO_5$
2,4-dinitrophenol	51-28-5	$C_6H_4N_2O_5$
5-nitrovanillin	6635-20-7	$C_8H_7NO_5$
4-nitrobenzenesulfonic acid	138-42-1	$C_6H_5NO_5S$
4-nitrophthalic acid	610-27-5	$C_8H_5NO_6$
2-methoxy-4,6-dinitrophenol	4097-63-6	$C_7H_6N_2O_6$
3,5-dinitrosalicylic acid	609-99-4	$C_7H_4N_2O_7$
dinoterb	1420-07-1	$C_{10}H_{12}O_5N_2$

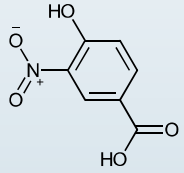
2-methoxy-4,6-dinitrophenol



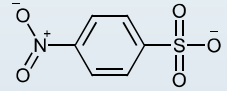
4-nitrocatechol



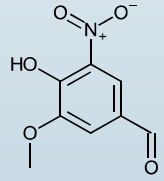
4-hydroxy-3-nitrobenzoic acid



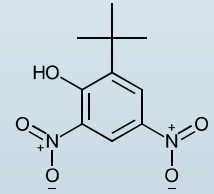
4-nitrobenzenesulfonic acid



5-nitrovanillin

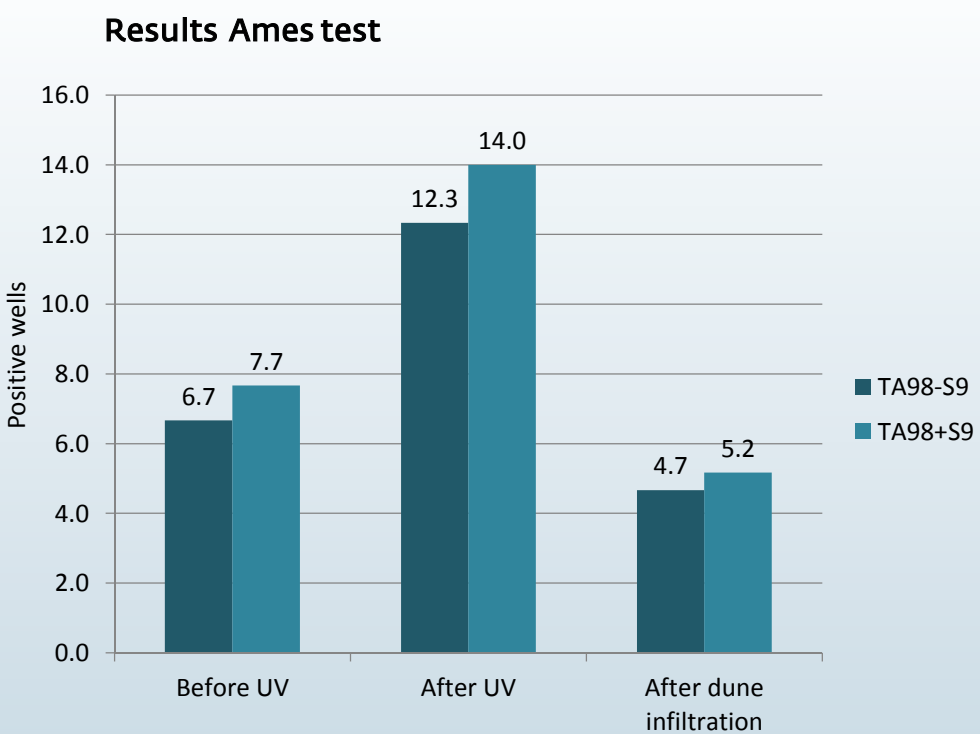


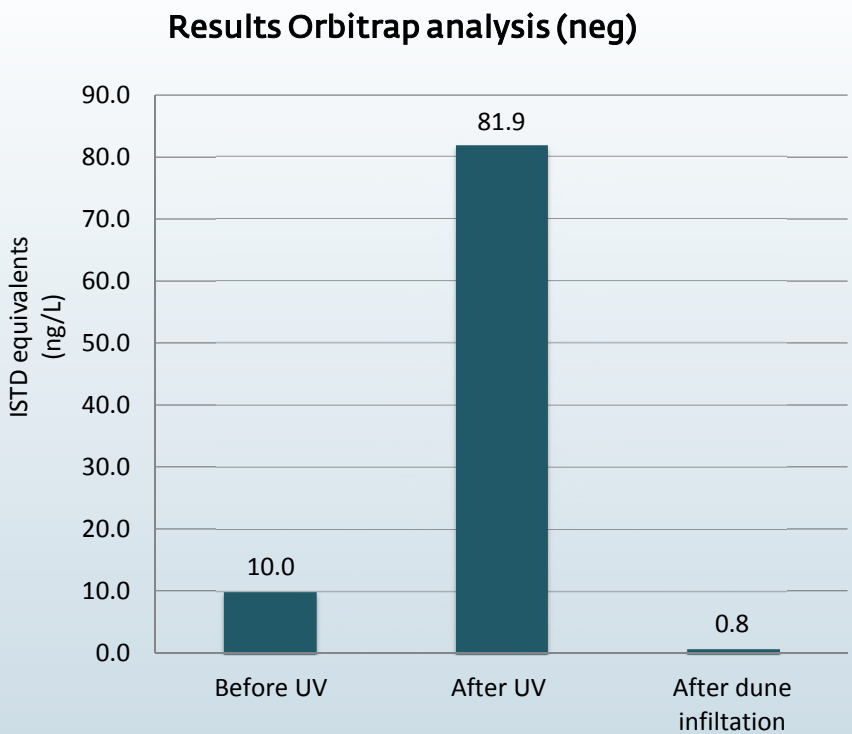
Dinoterb



Full scale water treatment

Results bioassays versus chemical analysis





Kolkman et al, Environ. Sci. Technol. 2015, 49, 4458.4465

Results

Genotoxic potential of identified N-DBPs

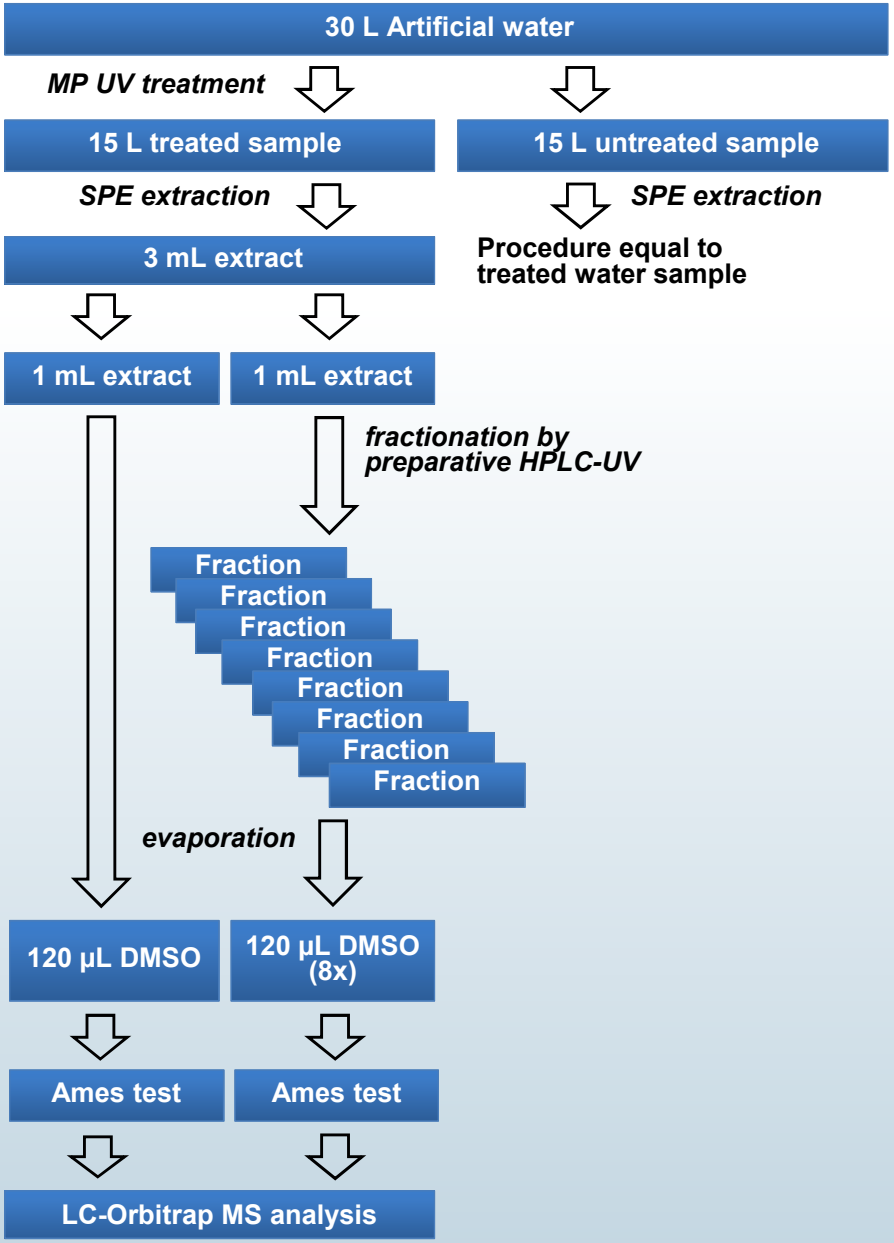
Compound	CAS nr	Formula	Genotoxic potential (based on measured data* and/or QSAR analysis)
4-nitrophenol	100-02-7	$C_6H_5NO_3$	Overall evidence points to absence of mutagenicity in Ames test; insufficient data to assess other genotoxicity and carcinogenic potential.*
4-nitrocatechol	3316-09-4	$C_6H_5NO_4$	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.

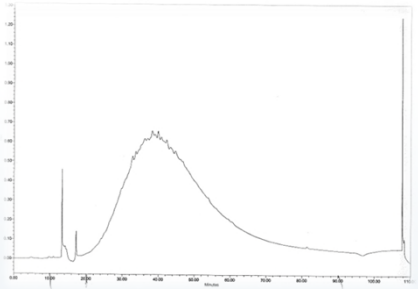
Effect directed analysis approach
Intro

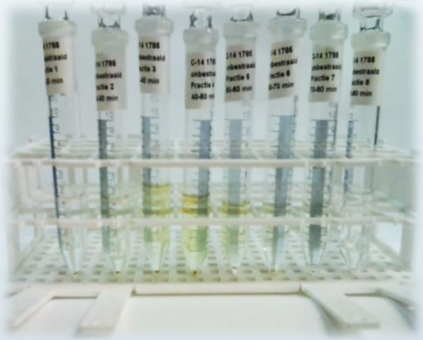
- **Genotoxic potential of the identified N-DBPs does not explain the observed Ames response**

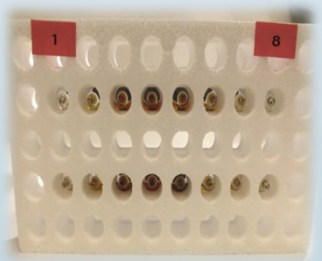
- **Application of effect directed analysis to identify mutagenic nitrogenous disinfection byproducts**
  - Preparative HPLC -> combining Ames mutagenicity testing and chemical screening results
  - Investigate which of the N-DBPs contribute to the mutagenic response

Experimental design



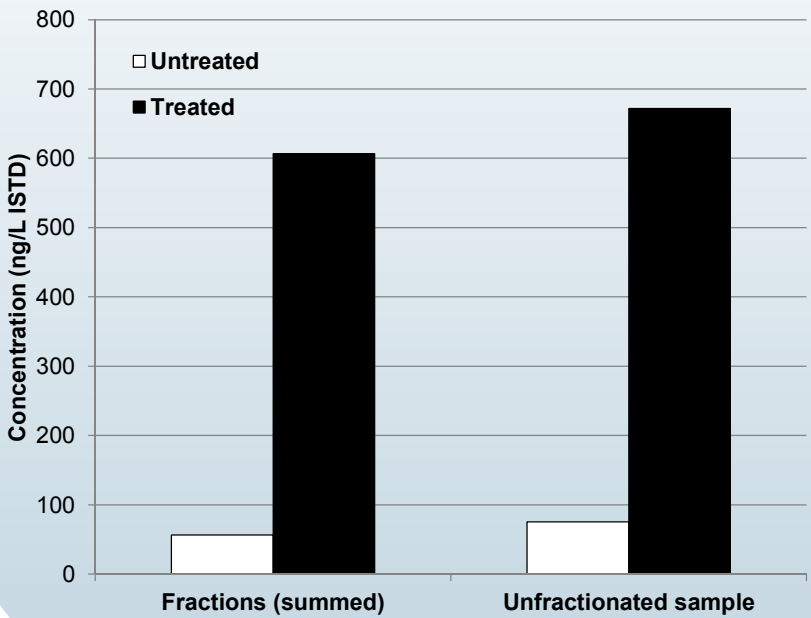




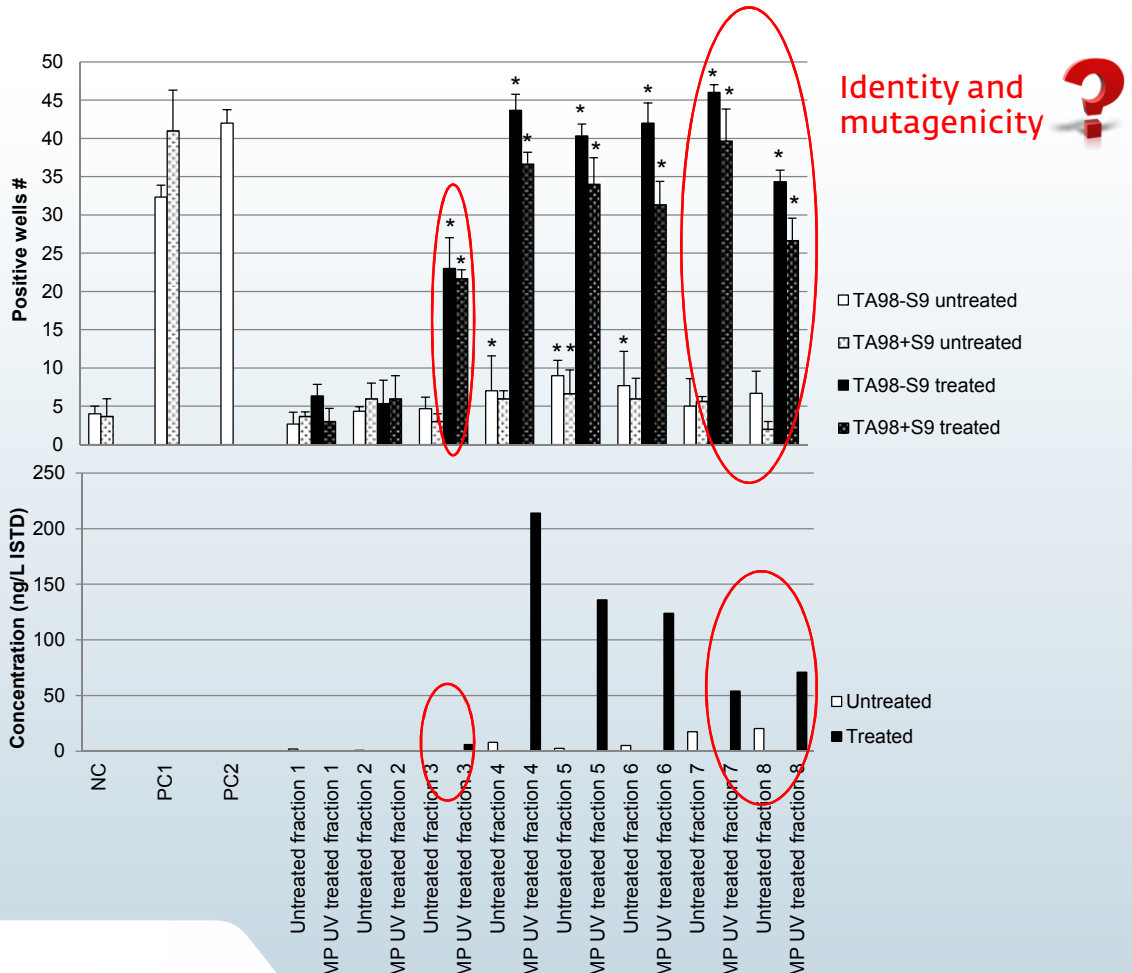


Fractionation and concentration of water extracts

- 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



N-DBPs in fractionated water extracts



Identity and mutagenicity

Top 5 of N-DBPs per fraction

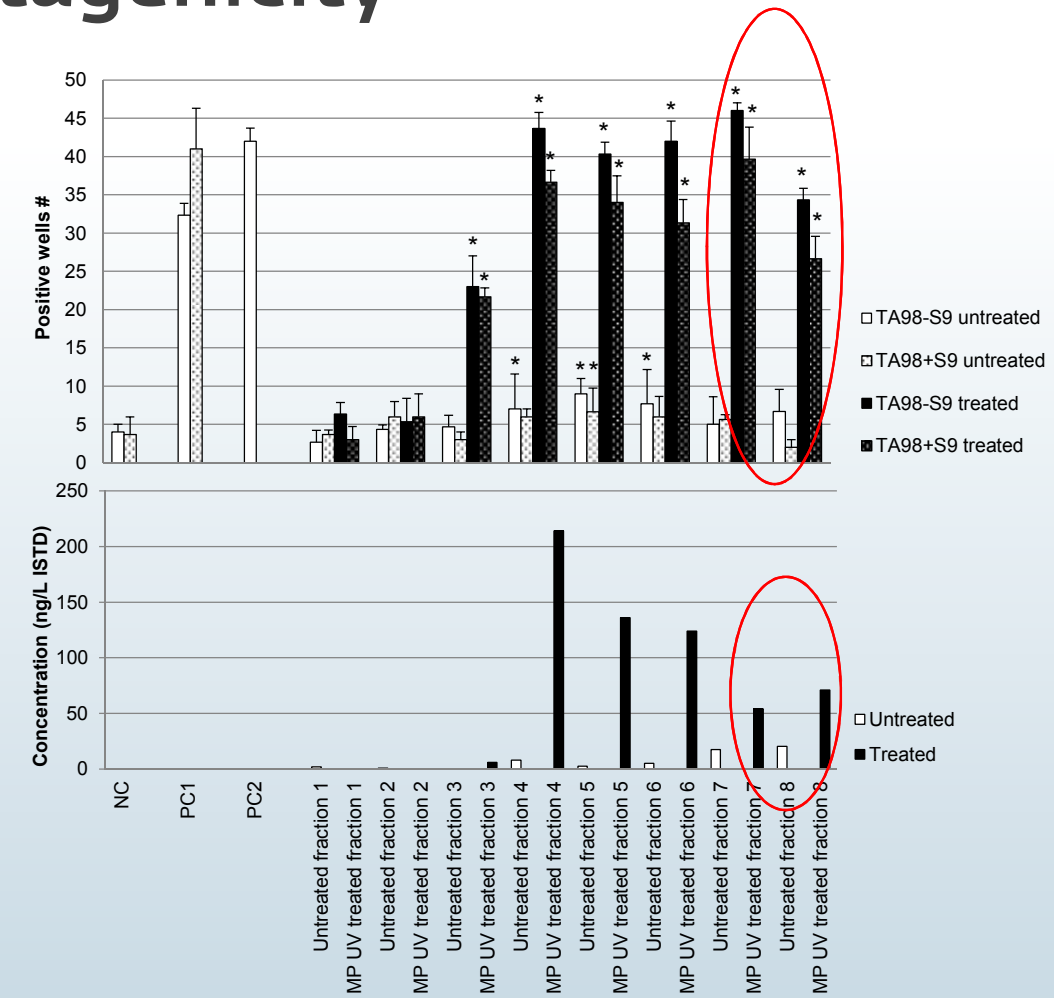
Mass (m/z)	Conc. (ng/L)	Formula	Compound
Fraction 3			
400.1262 (1)	1.9		
386.1096 (1)	1.3		
154.0148 (1)	0.8	$C_6H_5O_4N$	4-nitrocatechol
210.0048 (1)	0.7	$C_8H_5O_6N$	4-nitrophthalic acid
442.1365 (2)	0.4		
Fraction 4			
182.0098 (2)	42.2	$C_7H_5O_5N$	4-hydroxy-3-nitrobenzoic acid
138.0198	29.2	$C_6H_5O_3N$	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.

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	?	



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**
  - Identification and quantification of more by-products
  - Evaluation of the N-DBPs by more extensive QSAR and read across analysis and testing of (mixtures of) the N-DBPs in the Ames fluctuation tests

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