Application of Effect Directed Analysis to Identify Mutagenic Nitrogenous Disinfection Byproducts of Advanced Oxidation Drinking Water Treatment

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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))

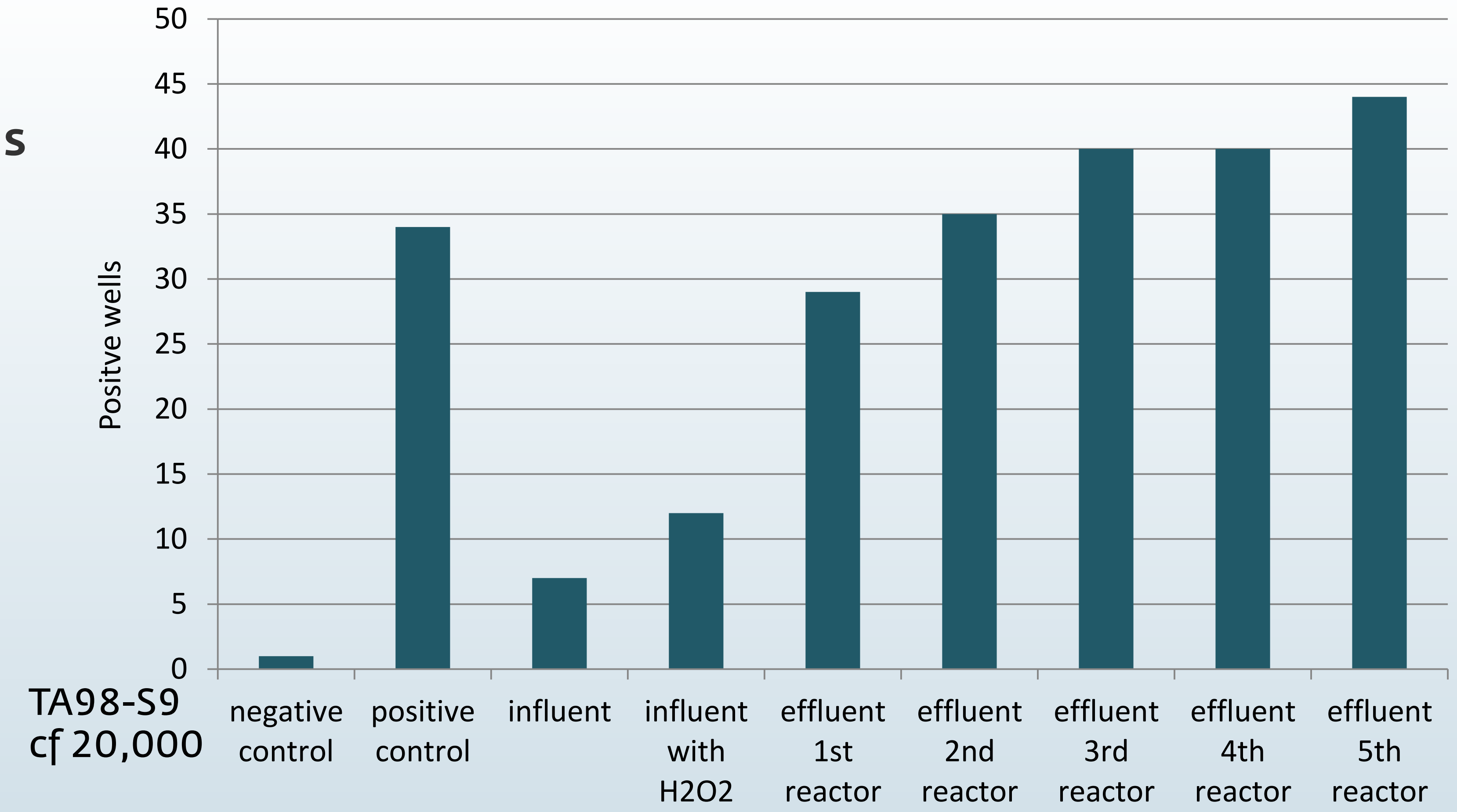
- **Water treatment may cause by-products**
  - THM's, HAA's (chlorination)
  - Bromate (ozone)
  - Nitrite (MP UV)

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?

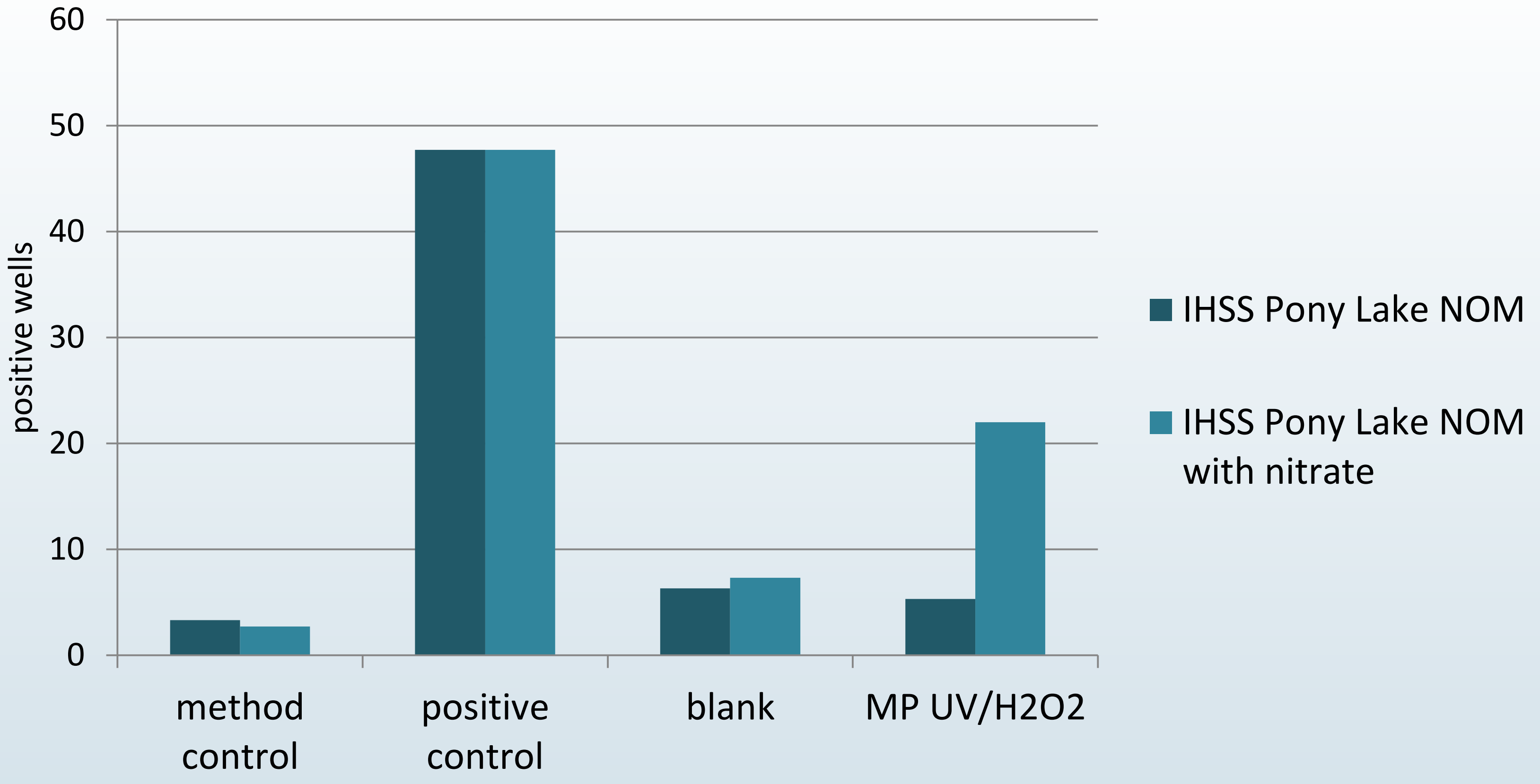
- probably caused by the formation of by-products



Source: PWN technologies

Introduction

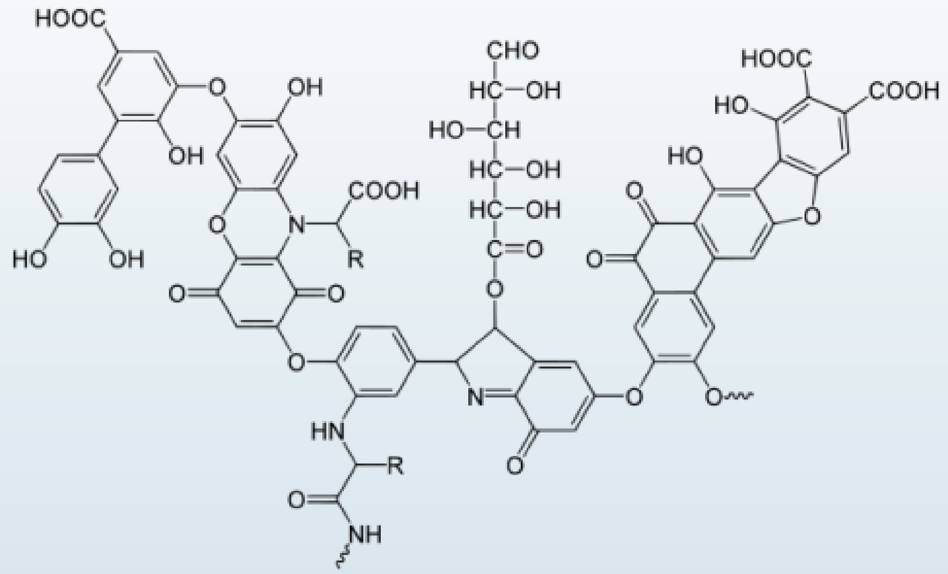
Ames response after MP UV/H_2O_2 treatment in artificial water



TA98-S9
cf 20,000

Source: PWN technologies

Example of humic acid



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

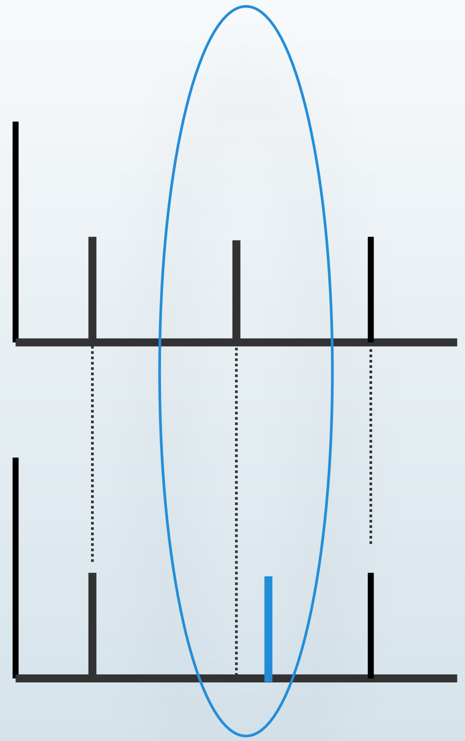
Introduction

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

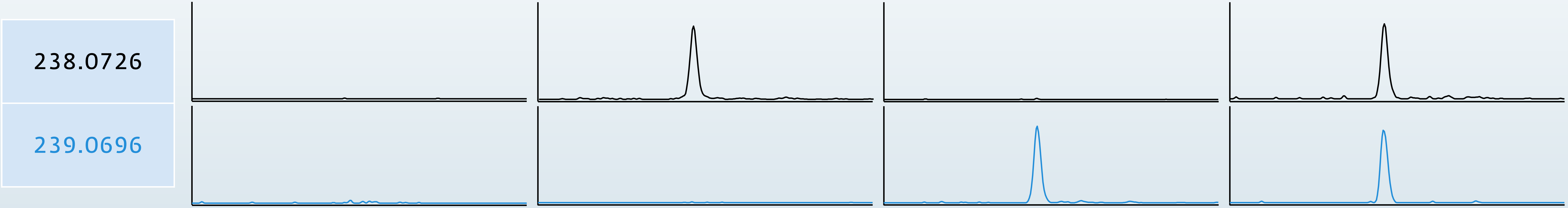
Δ m/z = 0.99704

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

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	-	+	+	+



- **84 detected byproducts**
- **14 compounds with 2x ^{15}N label**
- **Total concentration ≈1300 ng/L (ISTD eq.)**

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

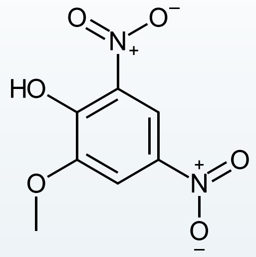
Kolkman et al, Environ. Sci. Technol. 2015, 49, 4458.4465

Results

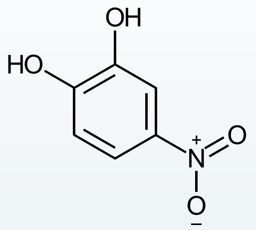
Identified N-DBPs - level 1, according to Schymansky

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	$C_{10}H_{12}O_5N_2$

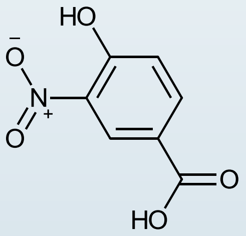
2-methoxy-4,6-dinitrophenol



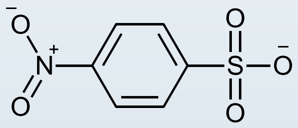
4-nitrocatechol



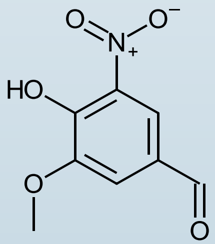
4-hydroxy-3-nitrobenzoic acid



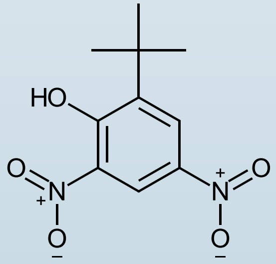
4-nitrobenzenesulfonic acid



5-nitrovanillin

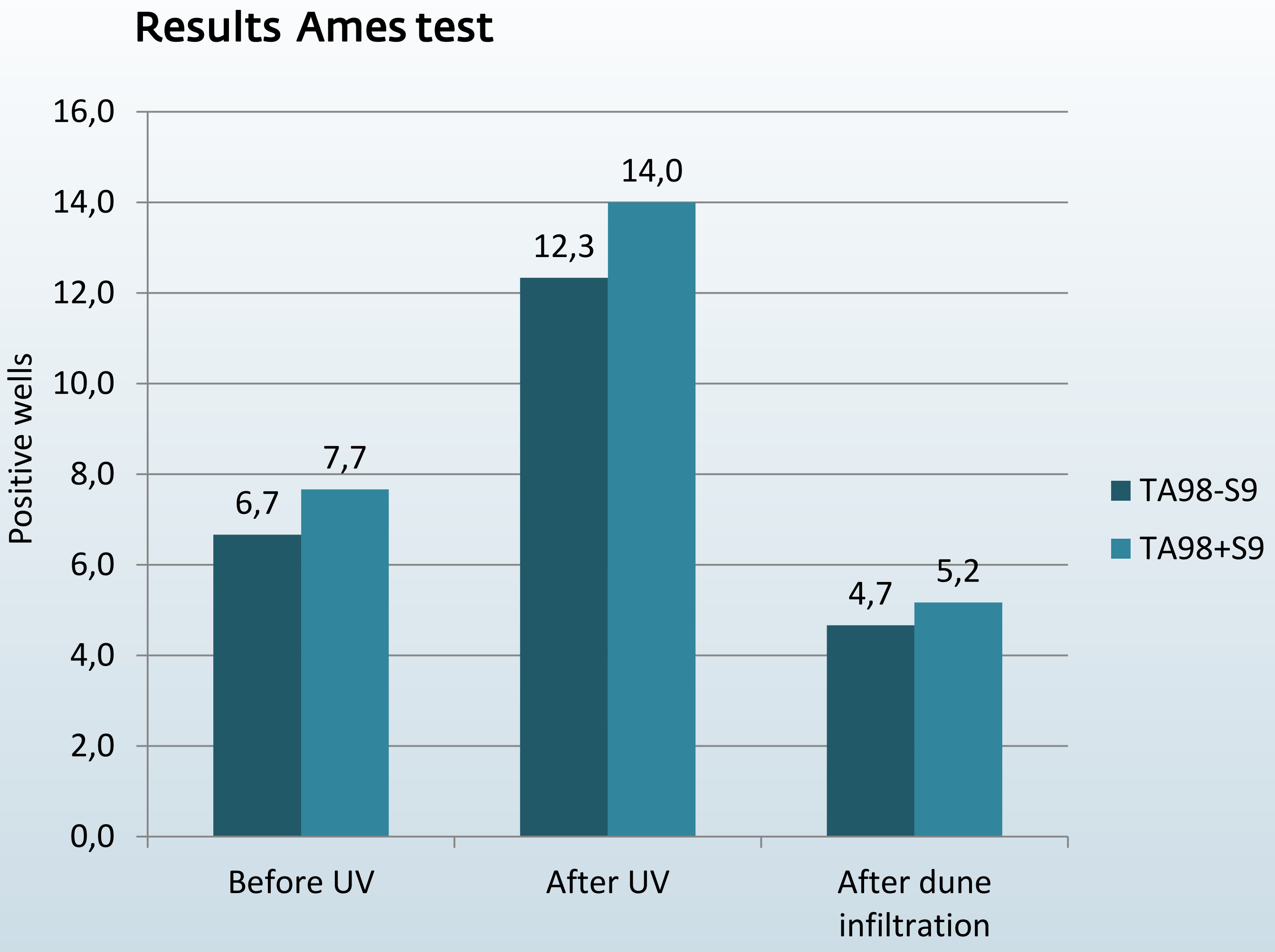


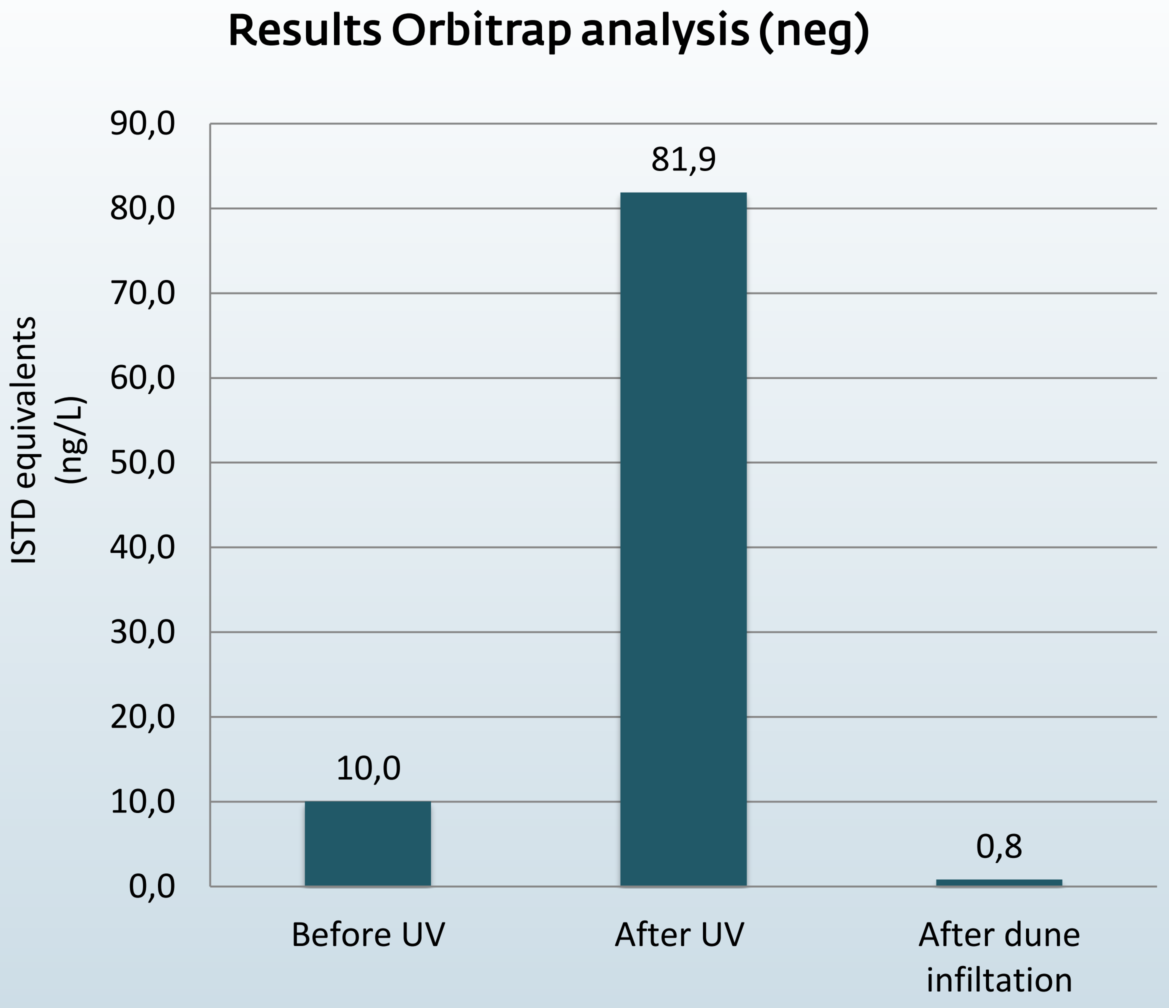
Dinoterb



Full scale water treatment

Results bioassays versus chemical analysis





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

Results

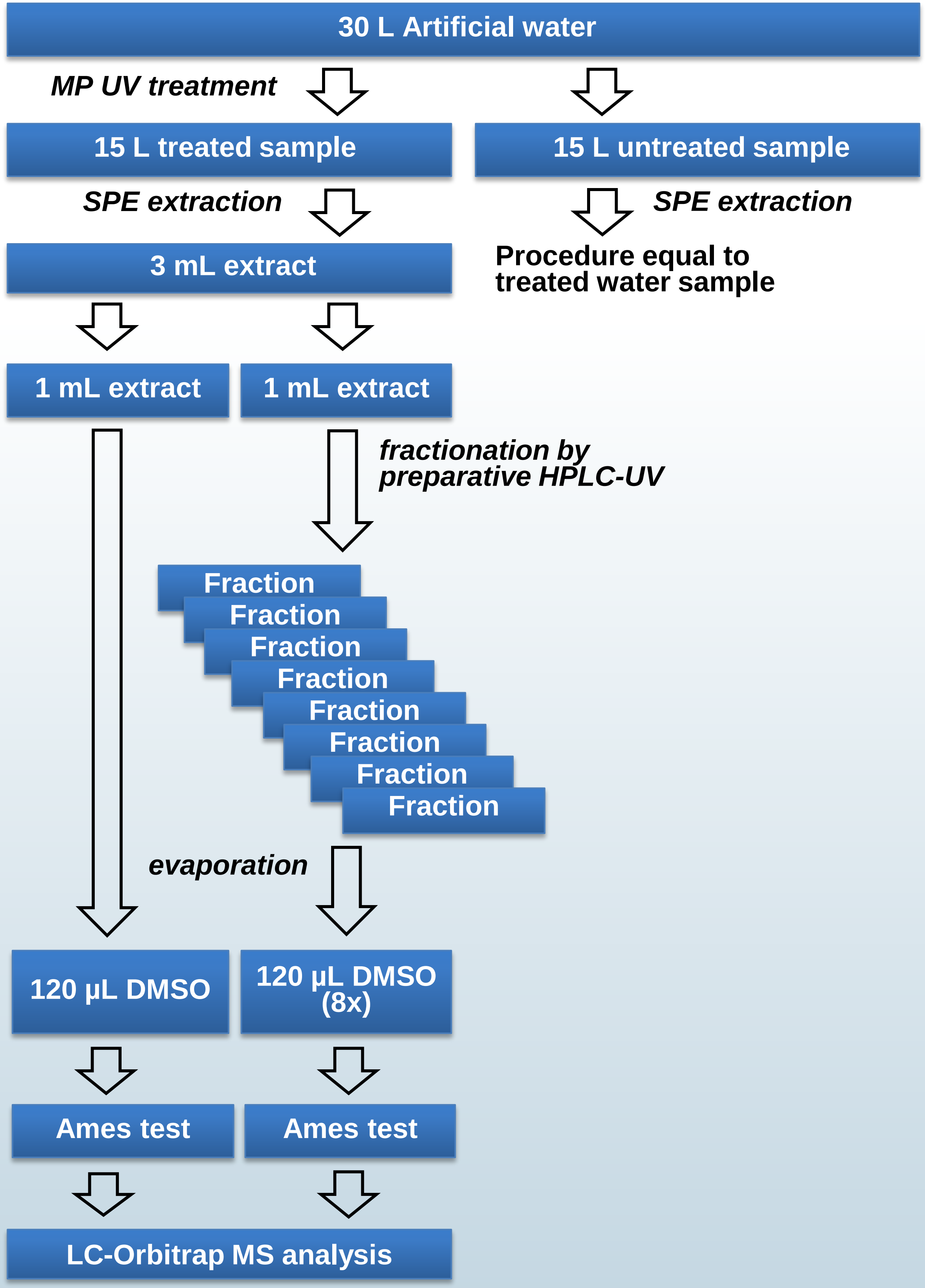
Genotoxic potential of identified N-DBPs

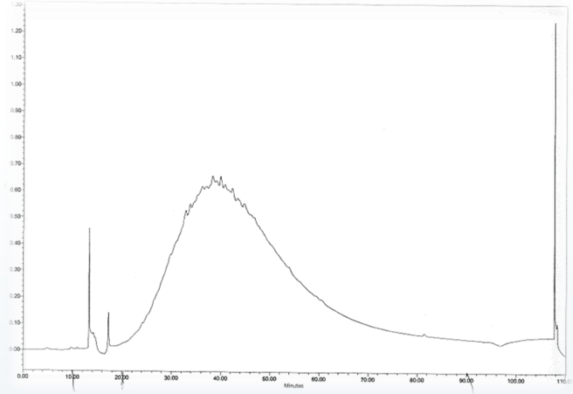
Compound	CAS nr	Formula	Genotoxic potential (based on measured data* and/or QSAR analysis)
4-nitrophenol	100-02-7	$C_6H_5NO_3$	*Overall evidence points to absence of mutagenicity in Ames test; insufficient data to assess other genotoxicity and carcinogenic potential.**
4-nitrocatechol	3316-09-4	$C_6H_5NO_4$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

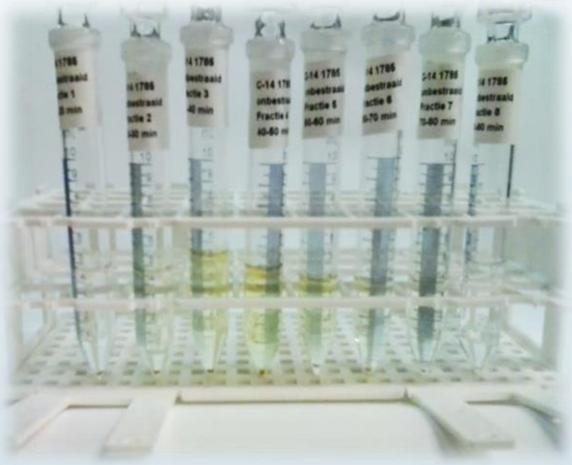
Effect directed analysis approach
Introduction

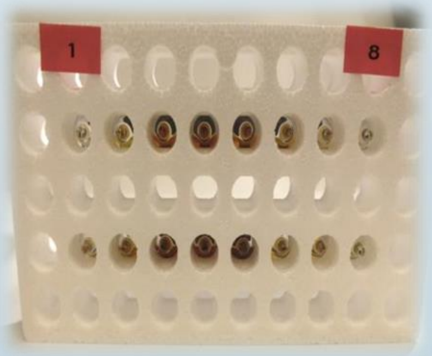
- **Genotoxic potential of the identified N-DBPs does not explain the observed Ames response**
- **Application of effect directed analysis to identify mutagenic nitrogenous disinfection byproducts**
  - Preparative HPLC -> combining Ames mutagenicity testing and chemical screening results
  - Investigate which of the N-DBPs contribute to the mutagenic response

Experimental design

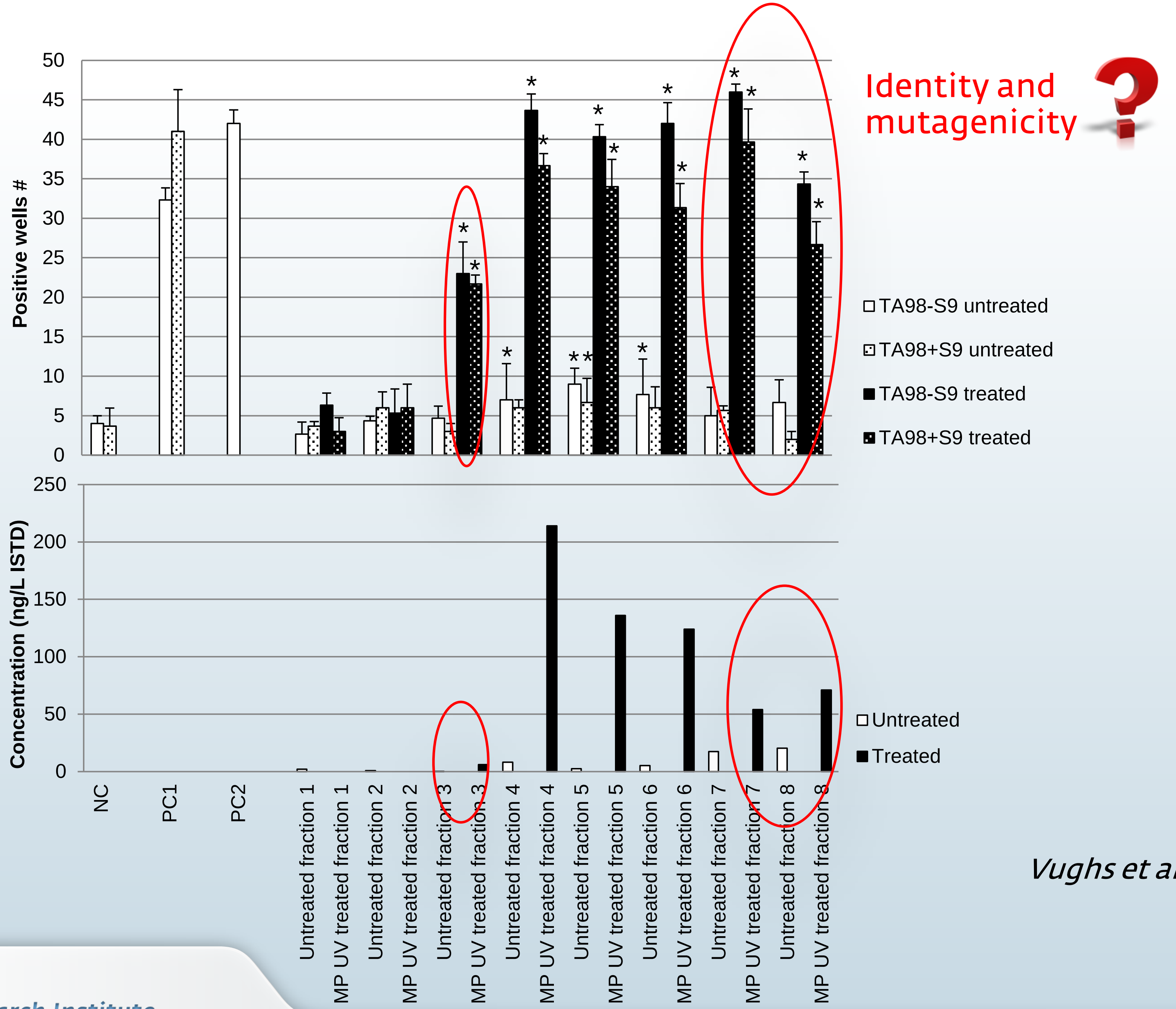








N-DBPs in fractionated water extracts



Vughs et al, Environ. Sci. Pollut.Res. 2016

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

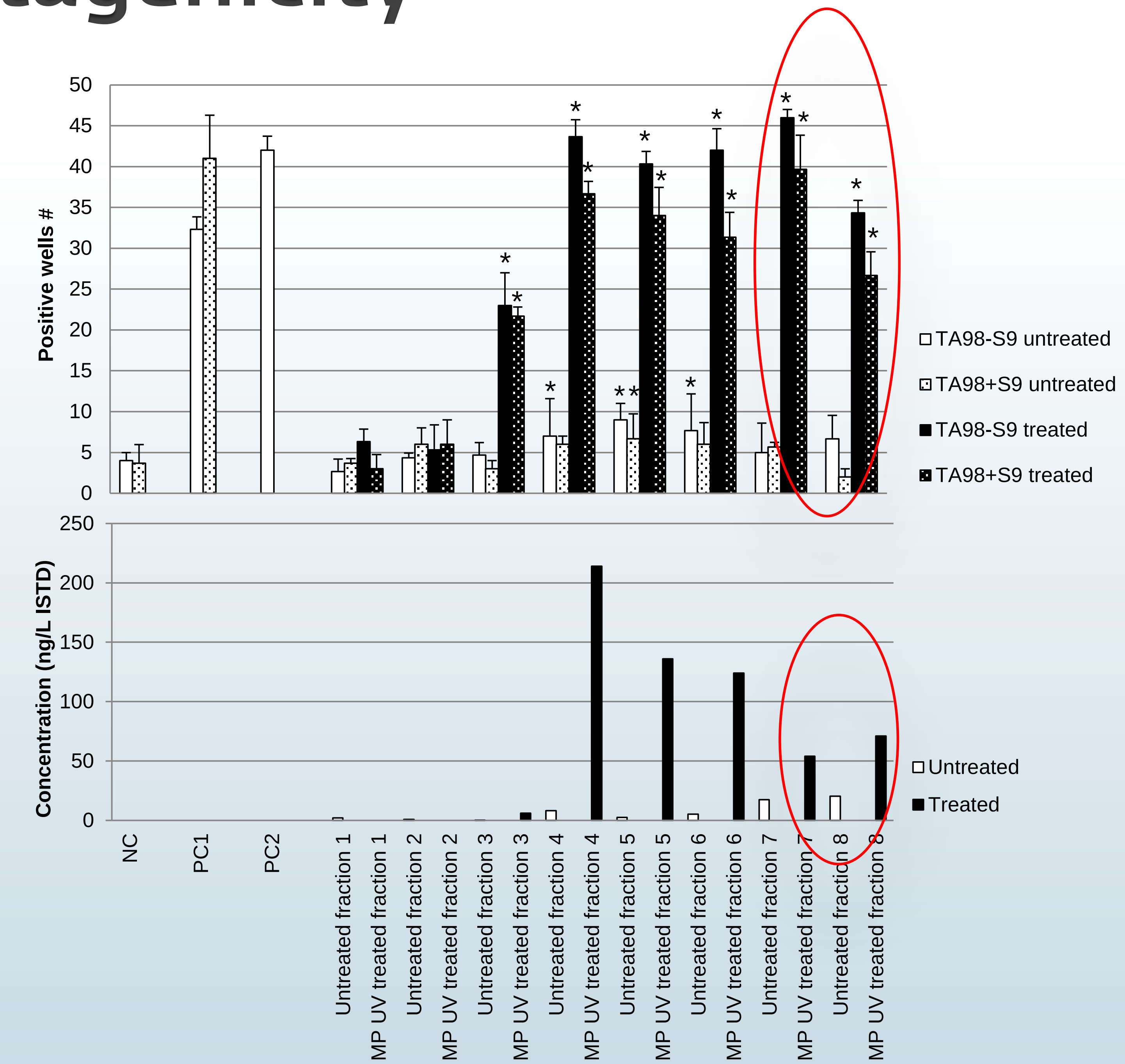
Mass (m/z)	Conc. (ng/L)	Formula	Compound
Fraction 6			
213.0154	38.5	$C_7H_6O_6N_2$	2-methoxy-4,6-dinitrophenol
316.1413 (3)	11.7	$C_{14}H_{23}O_7N$	
238.0726	9.0	$C_{11}H_{13}O_5N$	
270.0755 (1)	9.0		
316.1413 (1)	8.3	$C_{14}H_{23}O_7N$	
Fraction 7			
212.0204	23.9	$C_8H_7O_6N$	Structural isomer of 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	?	

Vughs et al, Environ. Sci. Pollut.Res. 2016



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
  - Using other analytical techniques (GC MS, HILIC, APCI, APPI) to analyze fractions
  - Labeling experiments with aromatic amino acids as precursors

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