Exploring the Great Unknown:
New Tools to Assess Complex Mixtures



NEMC, Orange County CA: 11th August 2016

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DOW

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Consumer Healthcare Products Association

CalgonCarbon

IXOM
Watercare

xylem
Let's Solve Water

GARVER

APE Research Council

Clean and Safe Water

Energy and Environment

Researchers find unsafe levels of industrial chemicals in drinking water of 6 million Americans

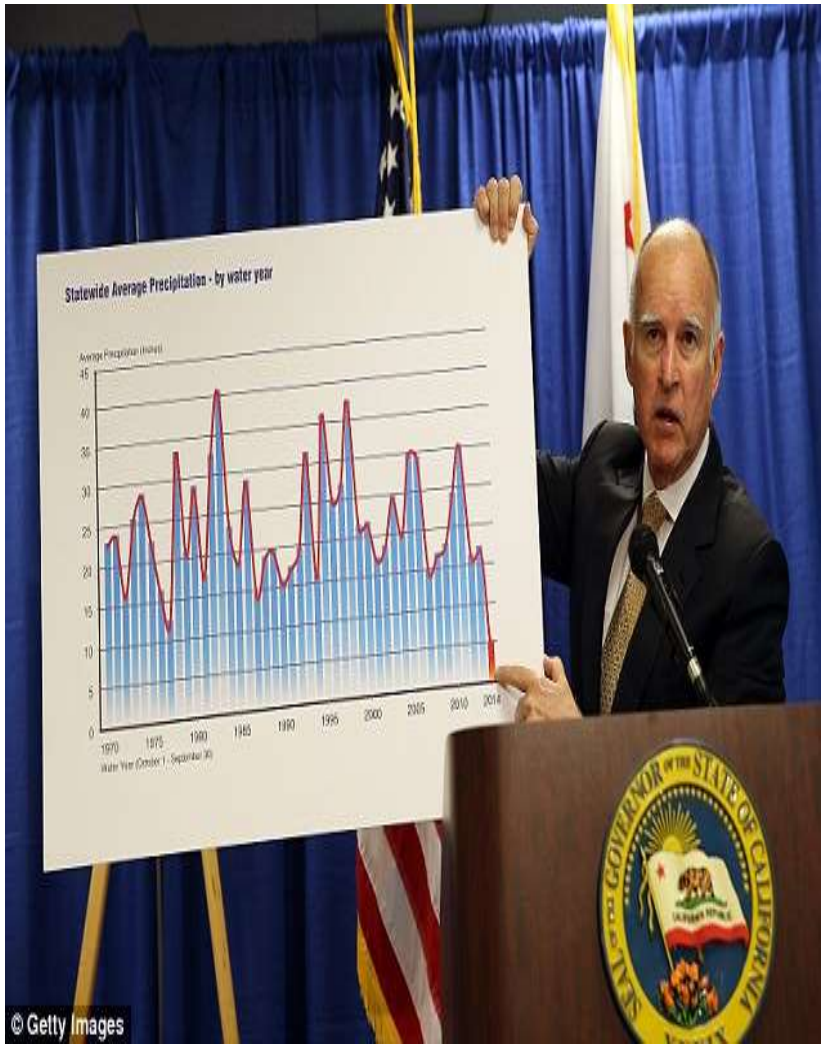
The Washington Post

Charges against 3 in Flint water crisis 'only the beginning'

Wed April 20, 2016

CNN

Water Reuse Future



OCT 08 2013 OFFICE OF THE GOVERNOR

To the Members of the California State Senate:

I am signing SB 322 which requires the Department of Public Health in consultation with the State Water Resources Control Board, to investigate the feasibility of developing uniform water recycling criteria for direct potable reuse by September 2016.

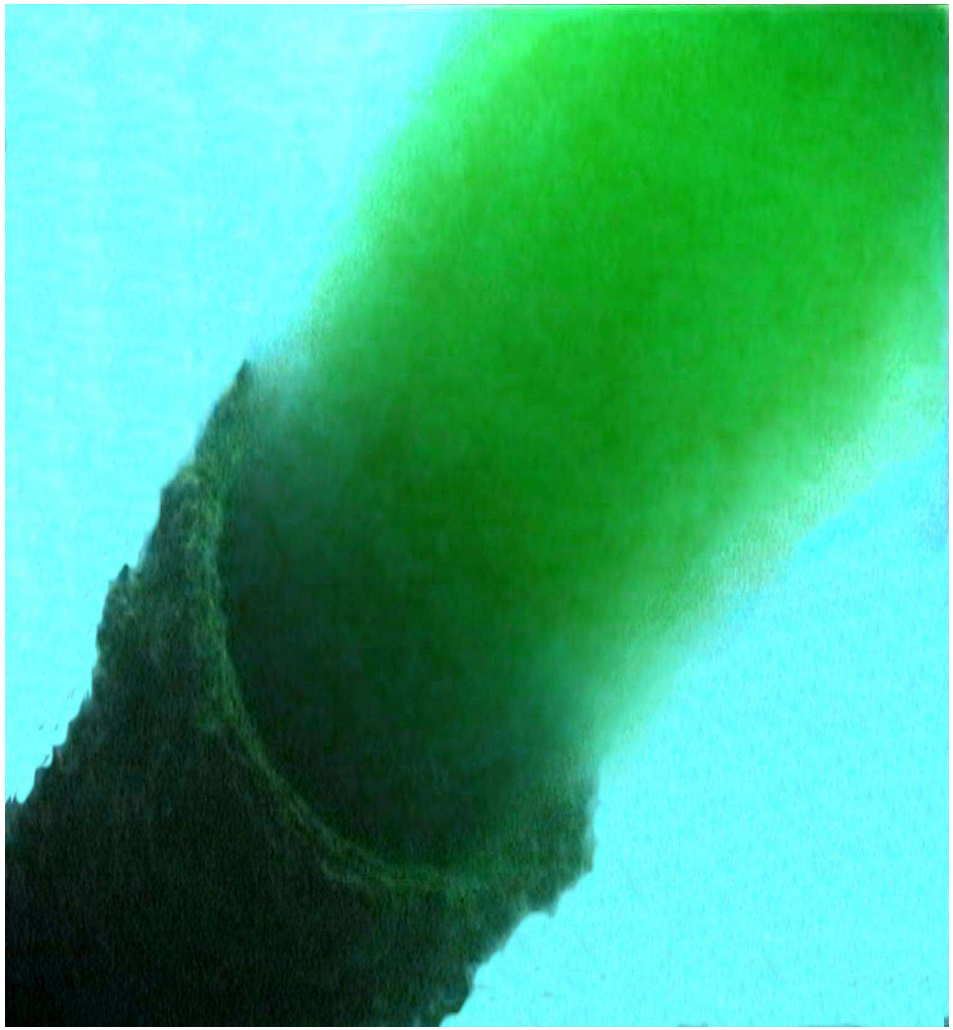
This information is past due. In an effort to enhance the use of recycled water, I have proposed the consolidation of the management of the drinking water program and all other water quality programs, including recycled water, under the State Water Board.

I am directing the Water Board to ensure that this work is completed expeditiously. The 3-year time frame mandated in this bill is too slow. California needs more high quality water and recycling is key to getting there.

Sincerely,

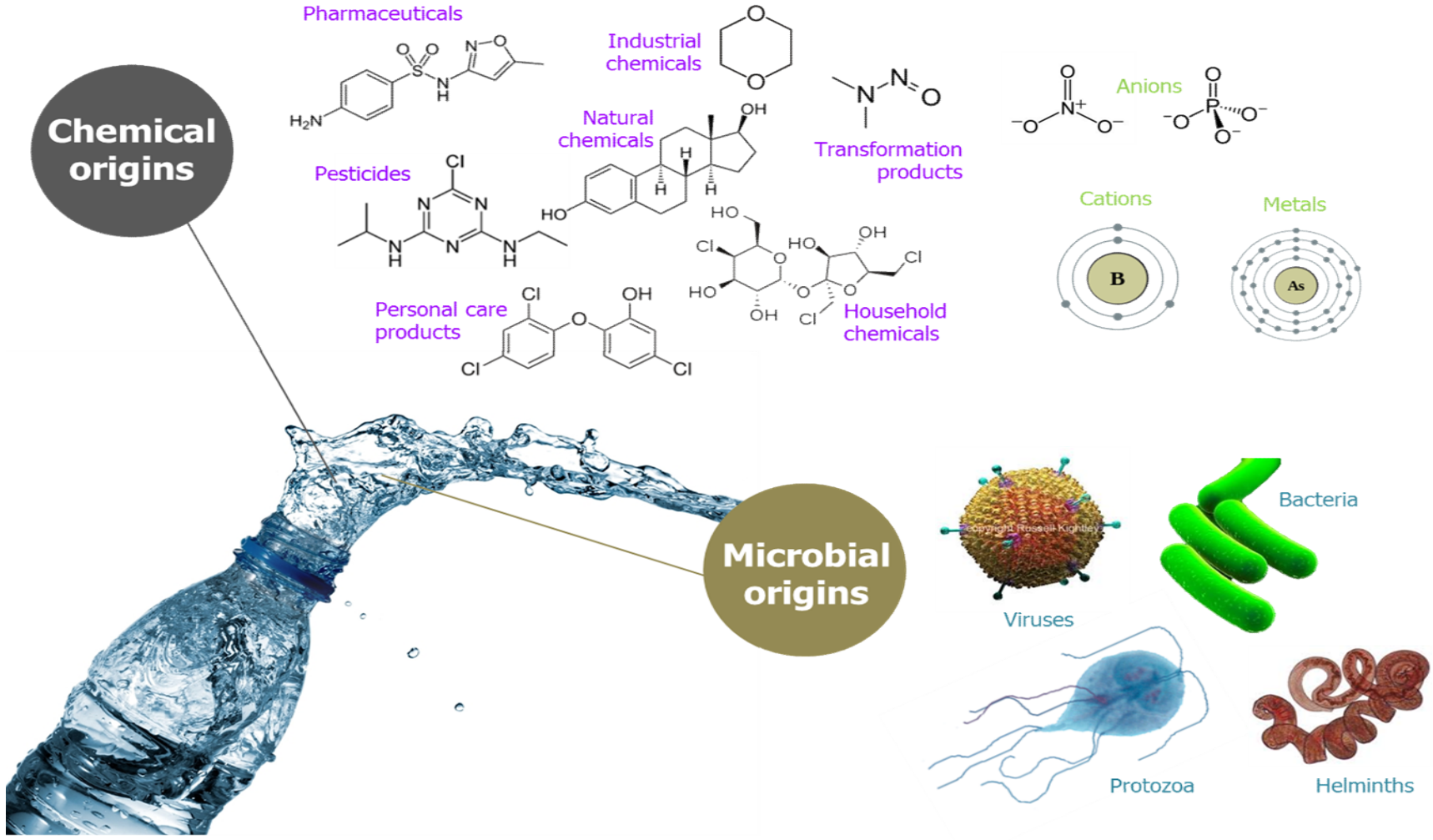
Edmund G. Brown Jr.

Restrictions on Ocean Outfalls



- **South Florida**
  - **2025 Ocean outfalls banned (except rain)**
- **Cape Cod**
  - **Moratorium on new outfalls and expanding existing**
- **California**
  - **Evidence of endocrine disruption at discharges**

Potential Contaminants



We Can Detect Anything, Anywhere

But are we looking for the right things?

azdailysun.com
Serving Flagstaff and northern Arizona

Is Flag's drinking water at risk?

CYNDY COLE Sun Staff Reporter | Posted: Tuesday, October 18, 2011 5:30 am

“About two years ago, very small traces of an antibiotic, an anti-seizure medication and a possible cancer-causing agent appeared in four groundwater wells in northwest Tucson.

All of the wells are located downstream of the local sewage treatment plant, which releases its treated sewage water into a riverbed.

When tested, some of Flagstaff's drinking water wells downstream of the Rio de Flag wastewater treatment plant have also shown tiny traces of other pharmaceuticals and hormones, which have an ability to influence growth in amphibians.”

IS IT SAFE?

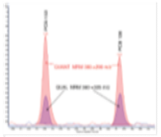
Comprehensive Screening

Targeted quantification

Analysis of a small set of predefined target compounds

Standards → Isolation and separation of analytes from matrix → Identification by RT & MS → Calculation of surrogate recoveries → Comparison to target peak areas → Quantification of specific compounds in a sample

Samples



LC-MS Triple Quad

Polar and moderately polar compounds

GC-MS Triple Quad

Volatile and semi-volatile compounds

ICP-MS

Metals and targeted organic complexes

IC-MS

Anions, cations, oxyhalides

Comprehensive Screening

Targeted quantification

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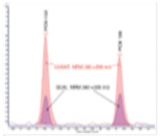
Samples



LC-MS Triple Quad

Polar and moderately polar compounds

GC-MS Triple Quad

Volatile and semi-volatile compounds

ICP-MS

Metals and targeted organic complexes

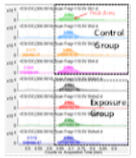
IC-MS

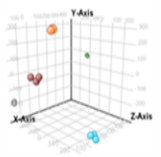
Anions, cations, oxyhalides

Non-targeted screening

Characterization of broader/unknown compounds

Sample → QTOF analysis and sample comparisons → Data alignment and analysis → Validation with MS/MS from standards → Compound profile in a sample





LC-MS QTOF

Polar and moderately polar compounds

GC-MS QTOF

Volatile and semi-volatile compounds

Cell Bioassay

Quantifiable cellular responses from mixtures isolated from biological samples

LC/GC-ICP-MS

Untargeted organic-halogens/ metals

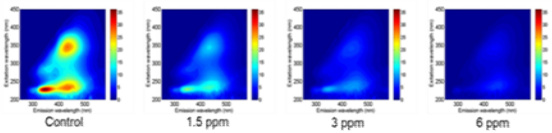
Tiered Testing Strategy

Tier 1

Bulk Water Characteristics (Surrogates)

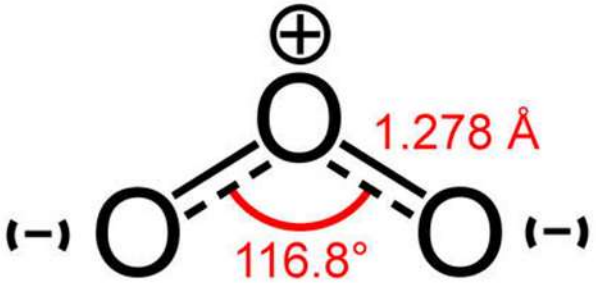
On-line & off-line analysis

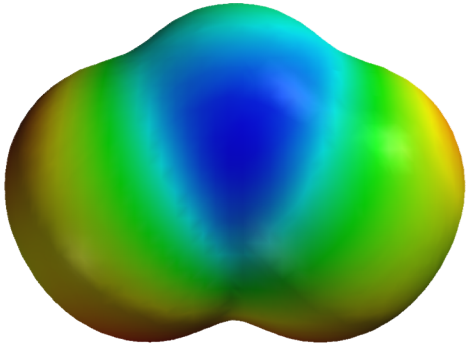
- General parameters (pH, temp, conductivity, turbidity, TSS)
- Organic parameters (TOC/DOC, UV254, fluorescence)
- Inorganic parameters (NO_3, NO_2, anion/cation, oxidant residuals)
- ***Near real-time performance***

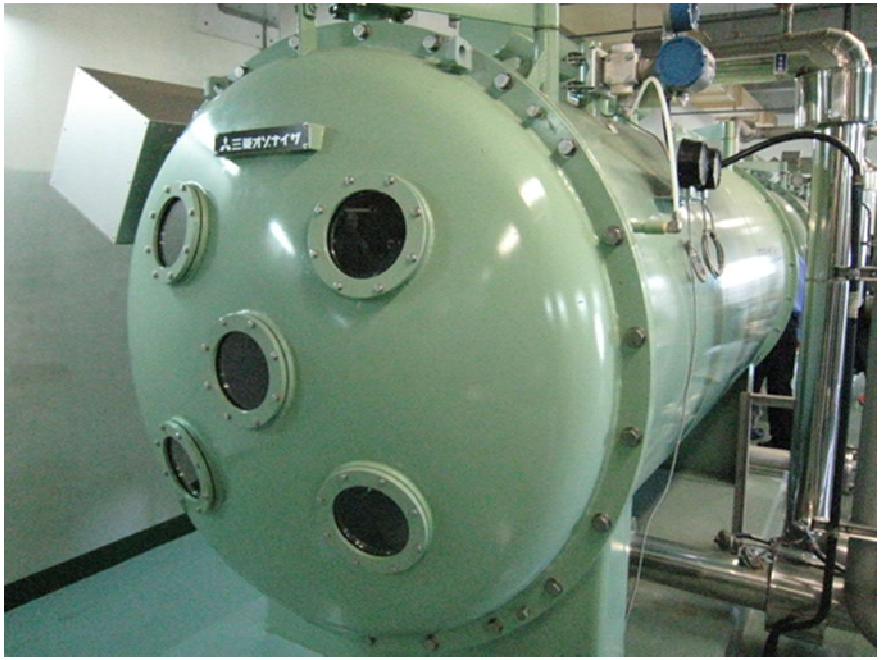


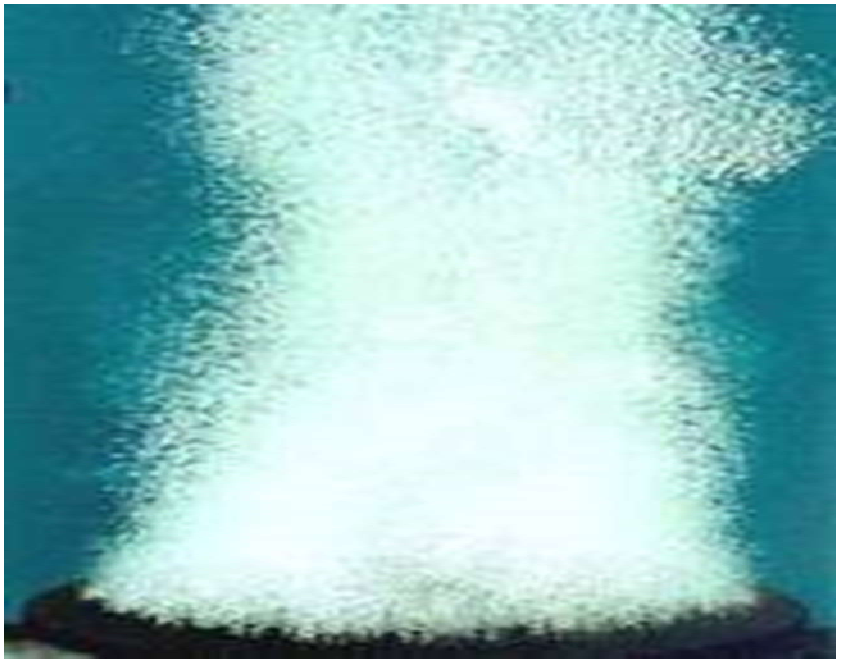
ftp://ftp.sccwrp.org/pub/download/DOCUMENTS/CECpanel/CECMonitoringInCARecycledWater_FinalReport.pdf

Ozone Oxidation

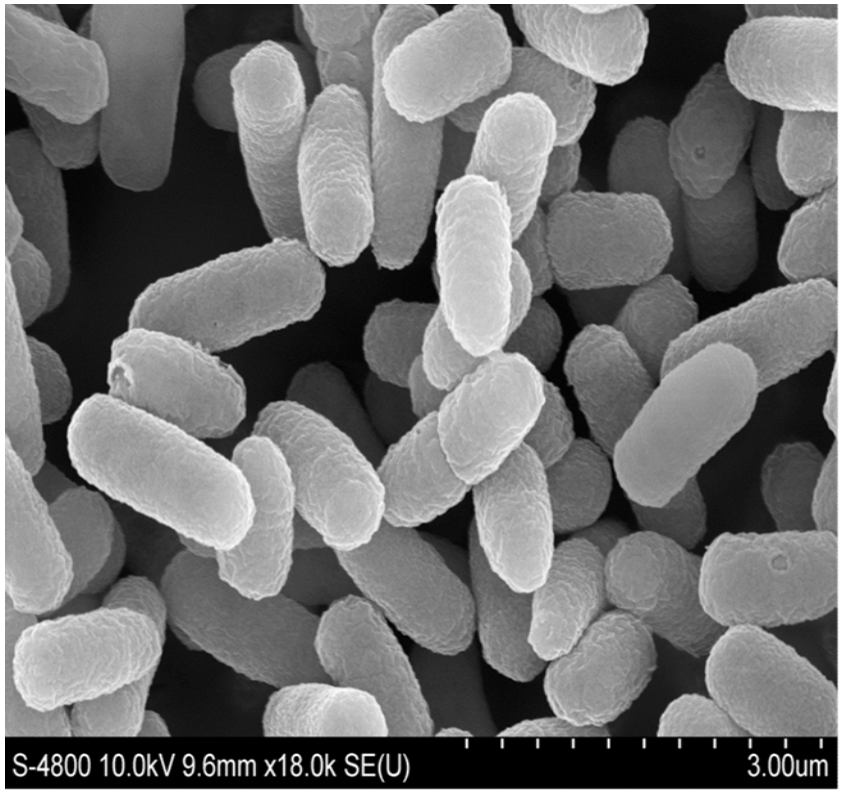




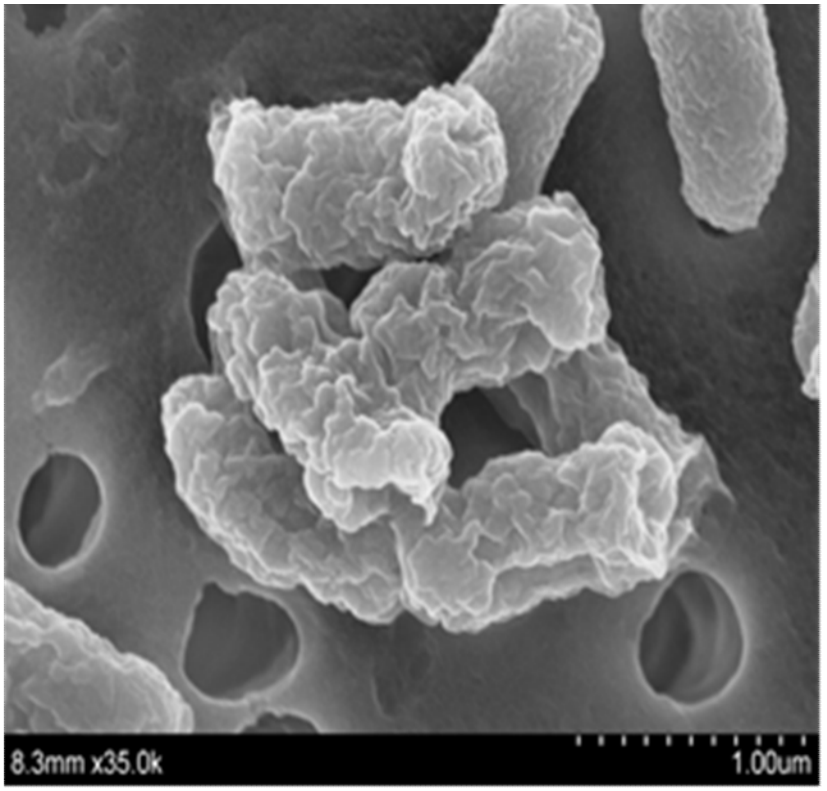




Ozone Disinfection



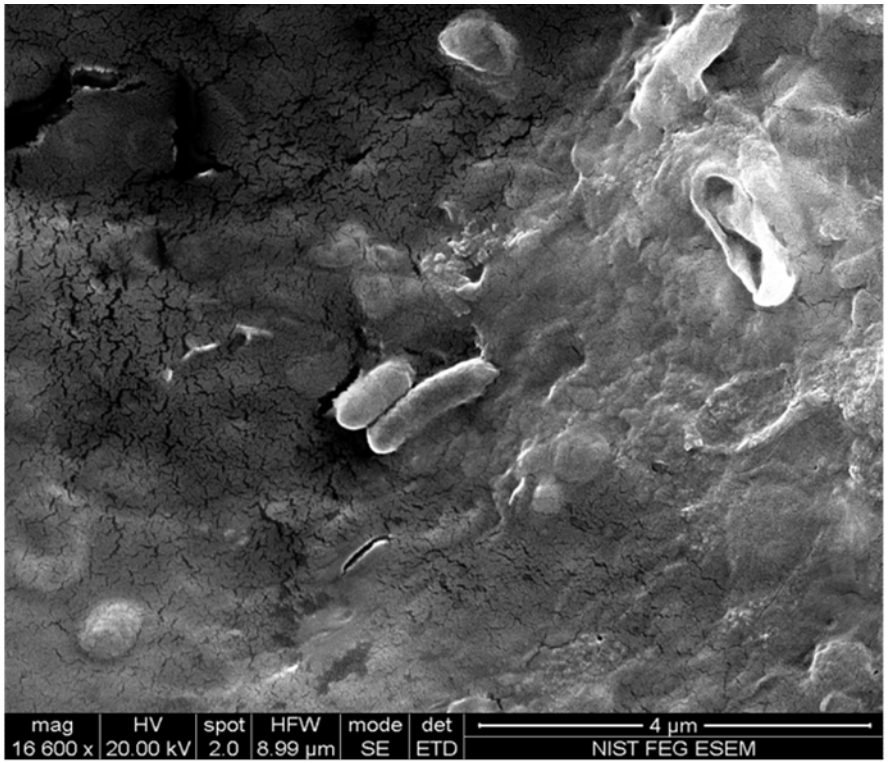
E. Coli - Healthy



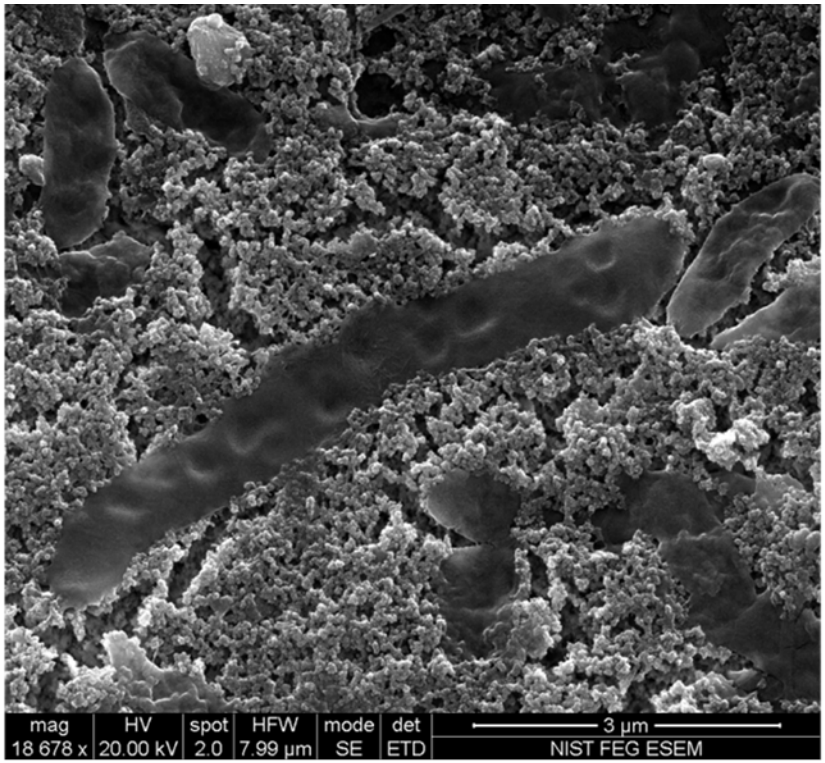
Post-AOP

Sherchan, S. P.; Snyder, S. A.; Gerba, C. P.; Pepper, I. L. *J. Environ. Sci. Health Part A-Toxic/Hazard. Subst. Environ. Eng.* **2014,** *49* (4), 397-403.

Membrane Fouling Reduction



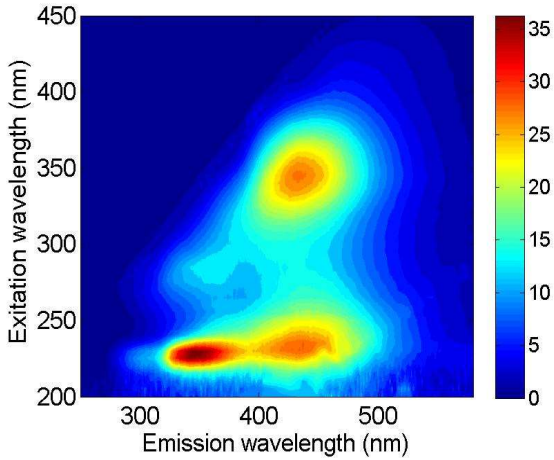
MBR-RO control



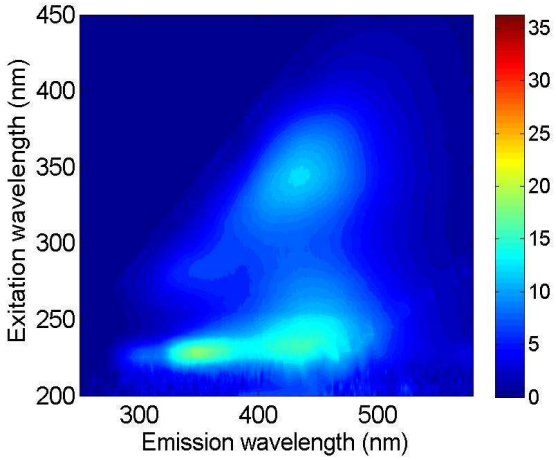
MBR-Ozone-RO (3 mg/L)

Stanford, B. D.; Pisarenko, A. N.; Holbrook, R. D.; Snyder, S. A., Preozonation Effects on the Reduction of Reverse Osmosis Membrane Fouling in Water Reuse. *Ozone-Sci. Eng.* **2011,** *33* (5), 379-388.

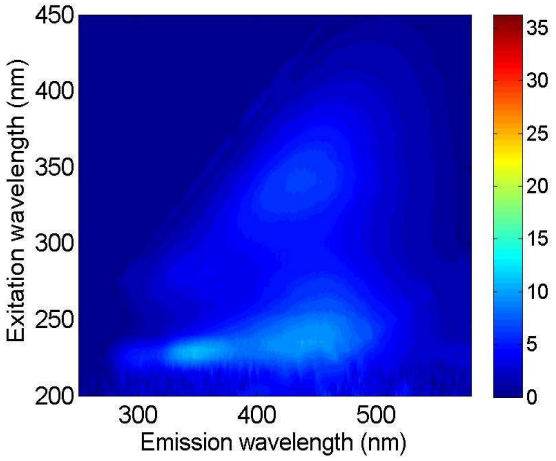
Fluorescence Surrogate



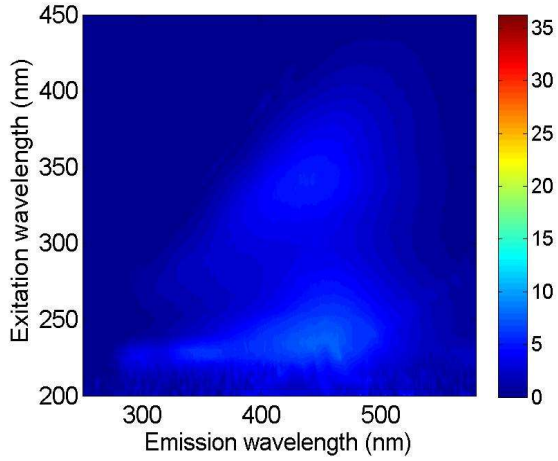
Control



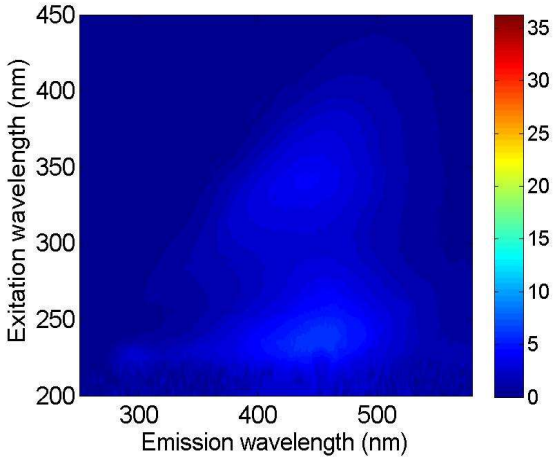
1.5 ppm



3 ppm



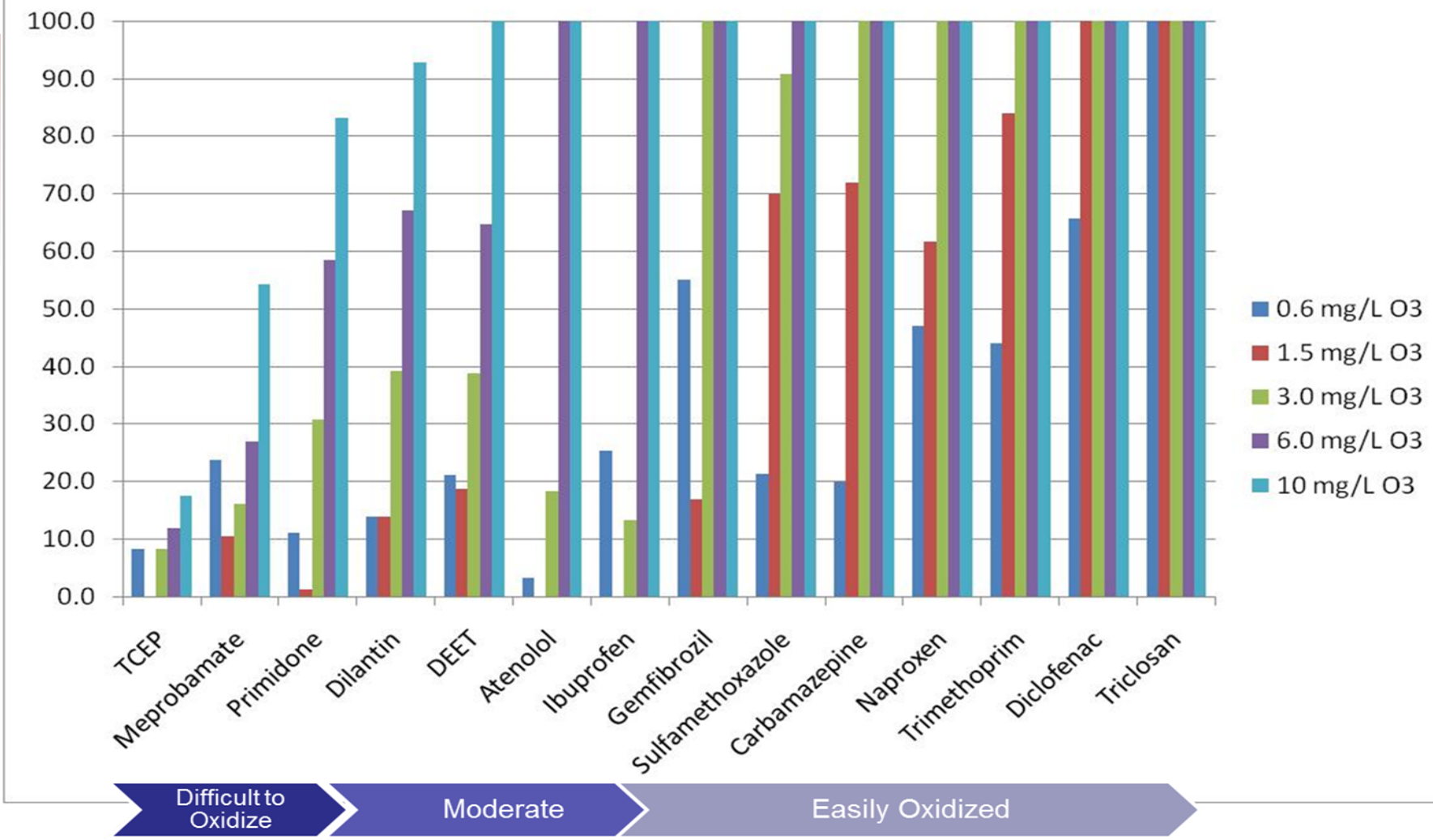
4.5 ppm



6 ppm

Merel S, Anumol T, Park M, Snyder SA. *J. Hazard. Mater.* 2015 282:75-85

Indicator: Ozone Oxidation



Pisarenko, AN et al.. *Water Res.* **2012,** *46* (2), 316-326.

Ozone Process Control

Environmental Science
Water Research & Technology

PAPER

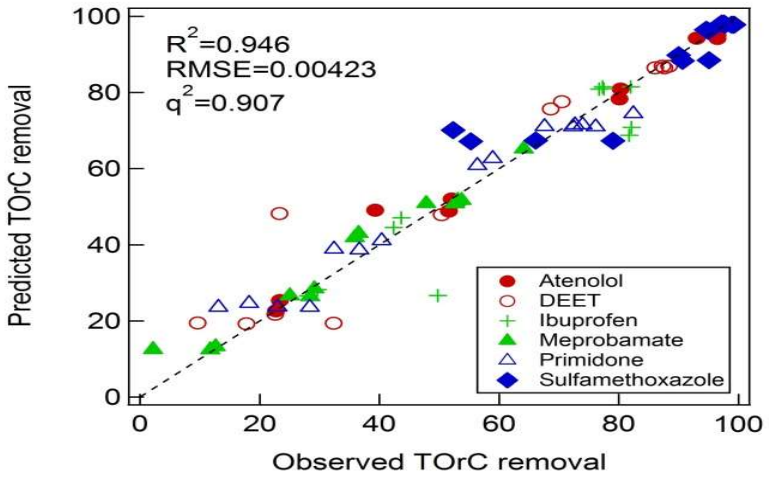
CrossMark

Cite this: *Environ. Sci.: Water Res. Technol.*, 2015, 1, 699

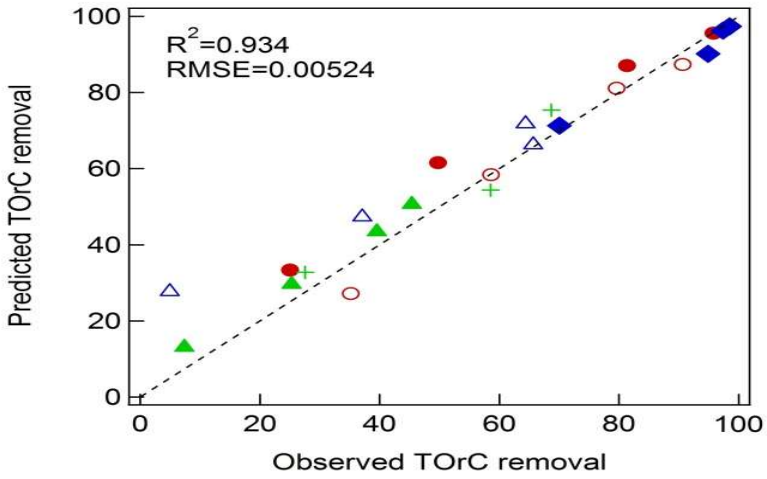
Modeling approaches to predict removal of trace organic compounds by ozone oxidation in potable reuse applications†

Minkyu Park,[a] Tarun Anumol[ab] and Shane A. Snyder*[ac]

PC-ANN: Generation/Training step



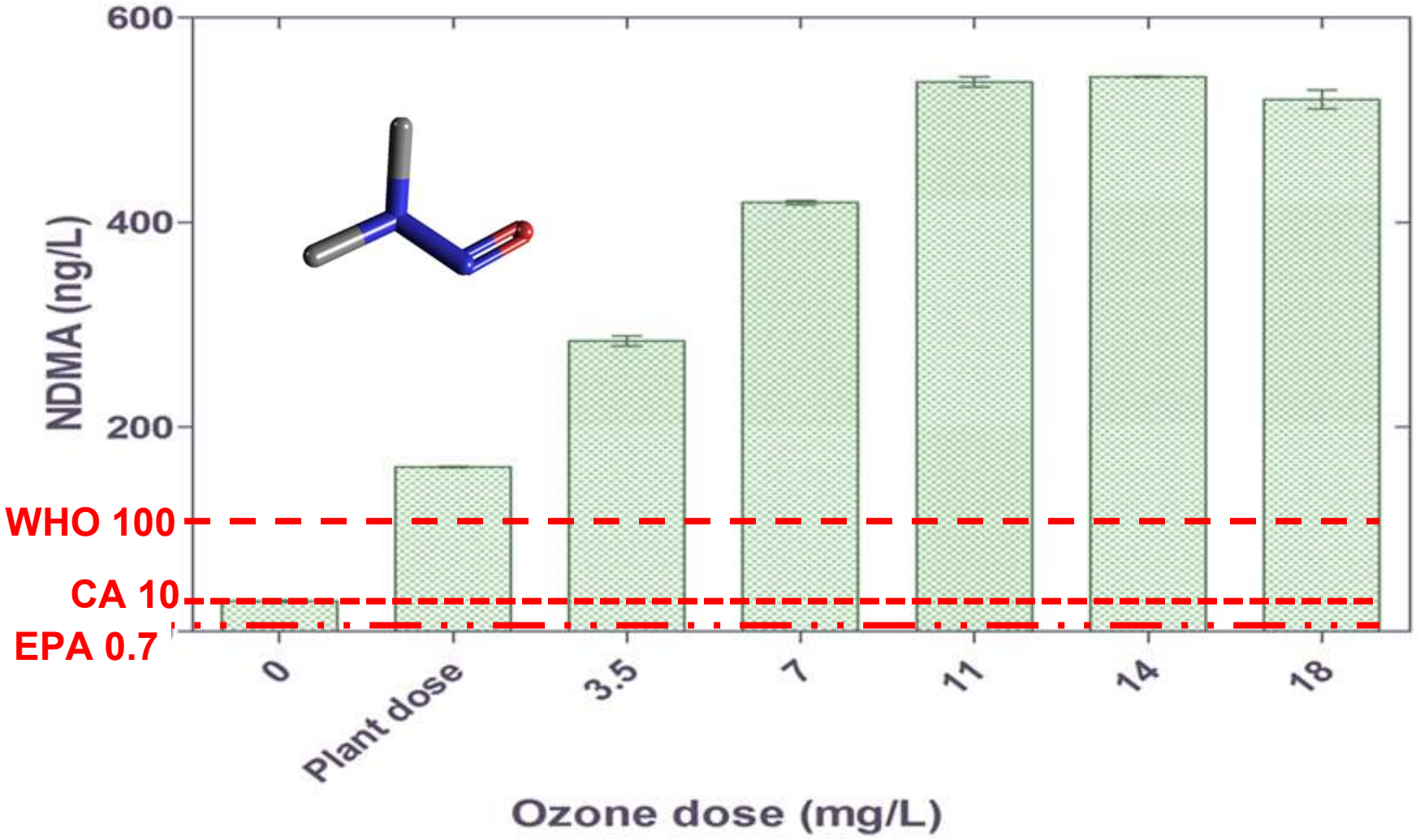
PC-ANN: External validation



On-Line/Real-Time Sensors

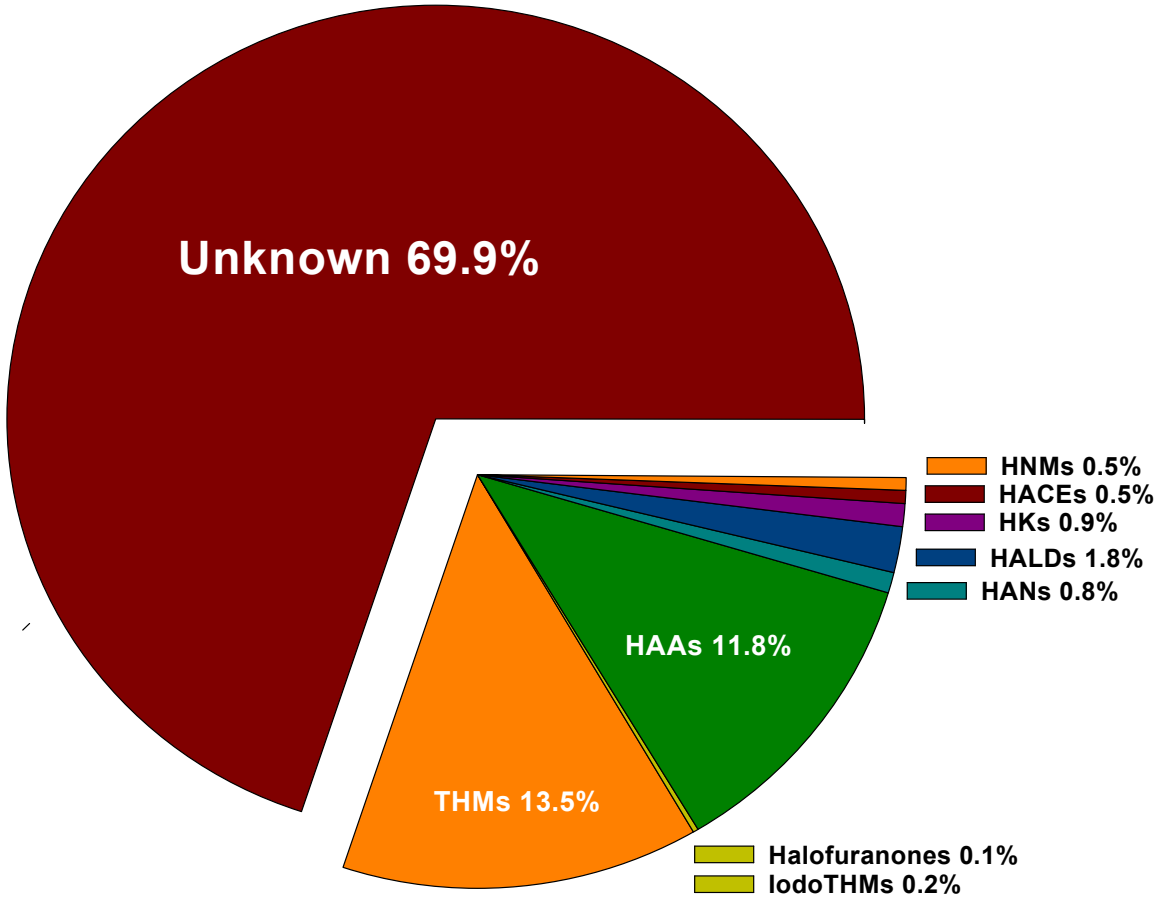
BUT, WHAT IS FORMED? DOC BEFORE OZONE AND AFTER OZONE IS ESSENTIALLY EQUAL.

NDMA Formation: Ozone



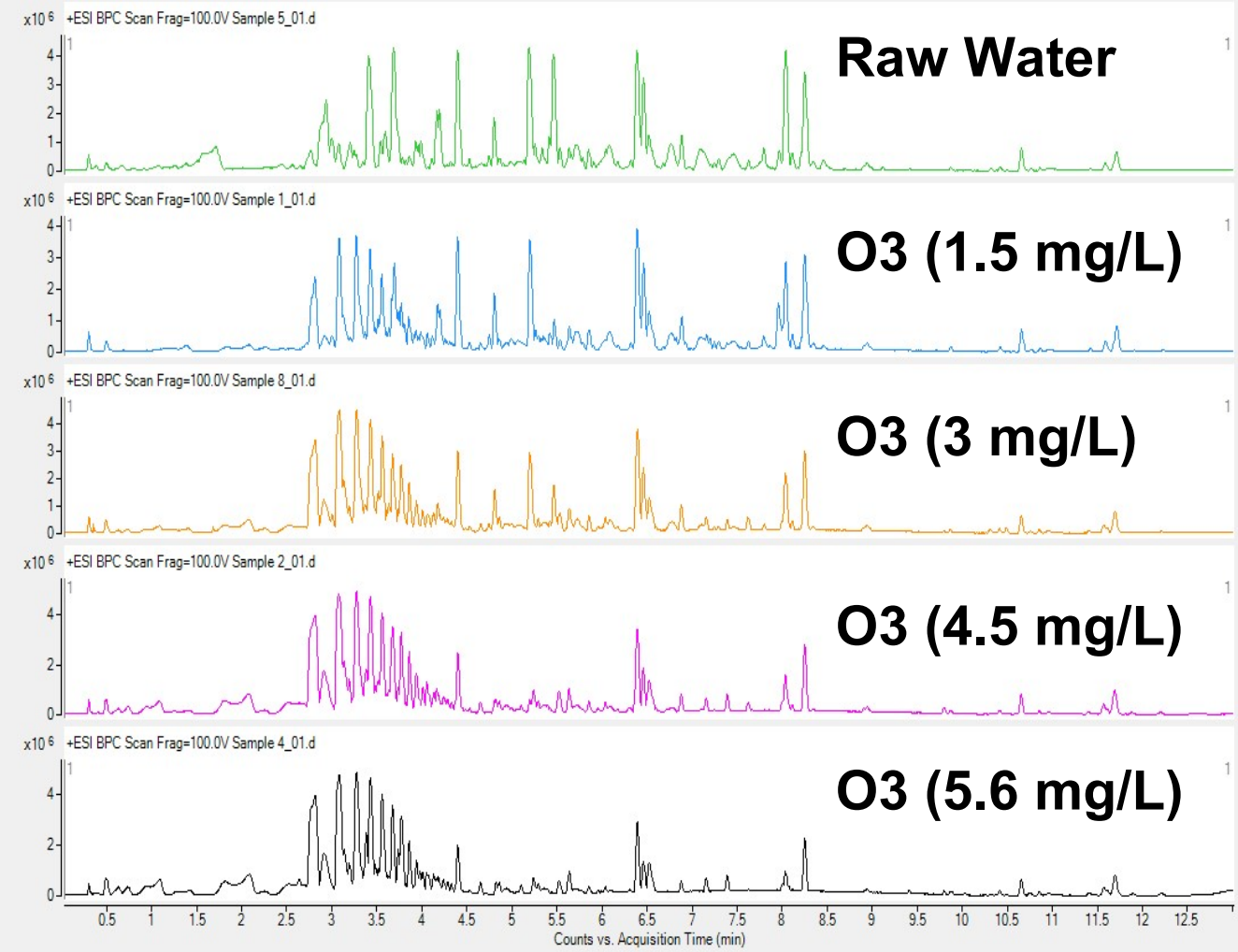
Sgroi M, Roccaro P, Oelker GL, Snyder SA. ***ES&T*** 2014 48:10308-10315.

Most DBPs Not Identified



Nationwide Occurrence Study, Krasner et al., *Environ. Sci. Technol*. 2006, *40*, 7175-7185.

OZONE TREATMENT & QTOF ANALYSIS OF UNKNOWNS



Chromatograms Very Similar

Extraction of Molecular Features Reveals thousands of compounds in each chromatogram

Further Data Processing Requires Specific Software

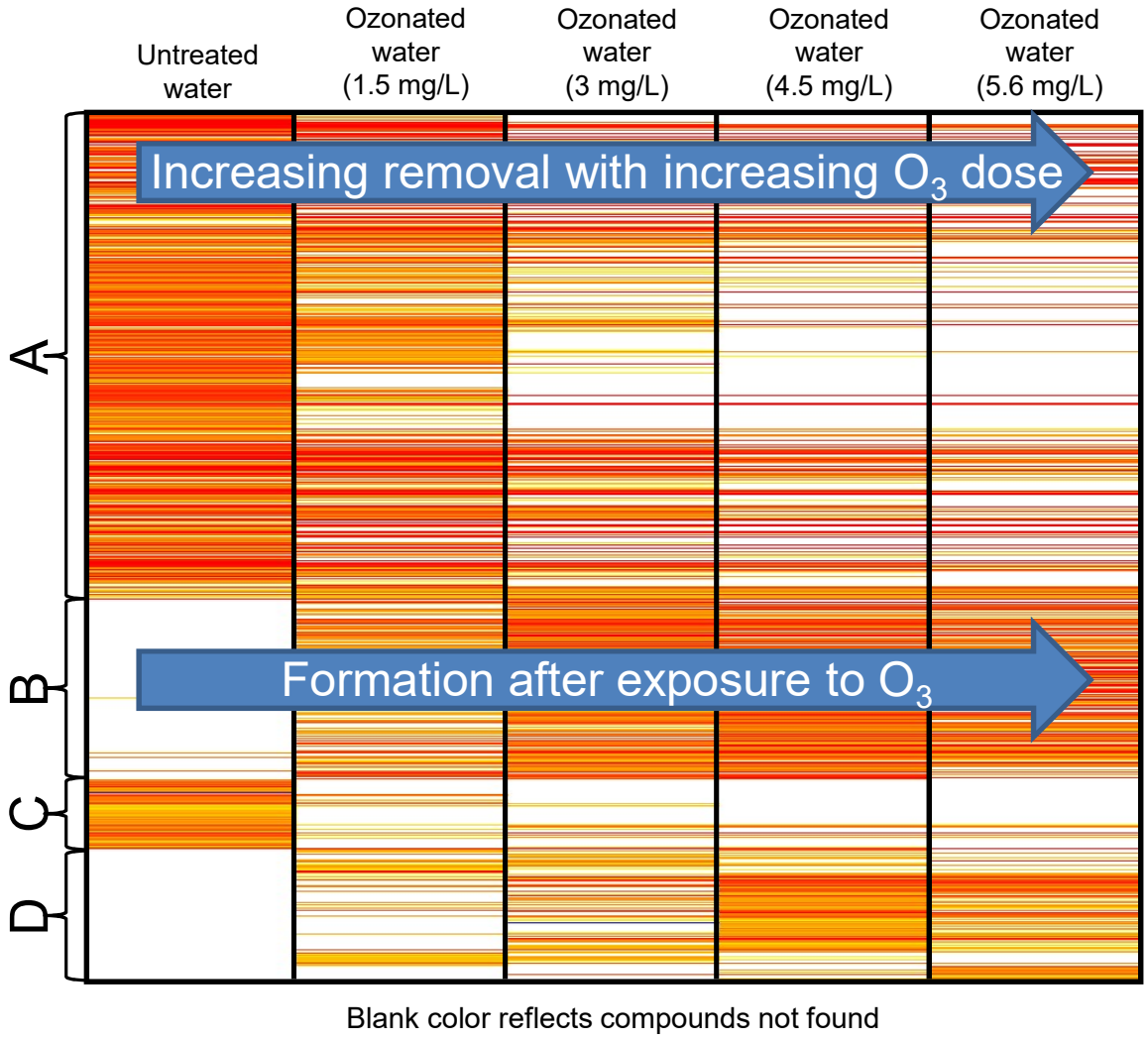
Merel S, Anumol T, Park M, Snyder SA. 2015 *J. Hazard. Mater.* 282:75-85

OZONE TREATMENT & QTOF ANALYSIS OF UNKNOWNS

Although chromatograms were all similar for the analyst, clear differences appear on the heatmap

A & C are group of compounds in the raw water but at lower concentration or absent in ozonated water **(removed by ozone)**

B & D are compounds absent in raw water but present in treated water **(ozone by-products)**



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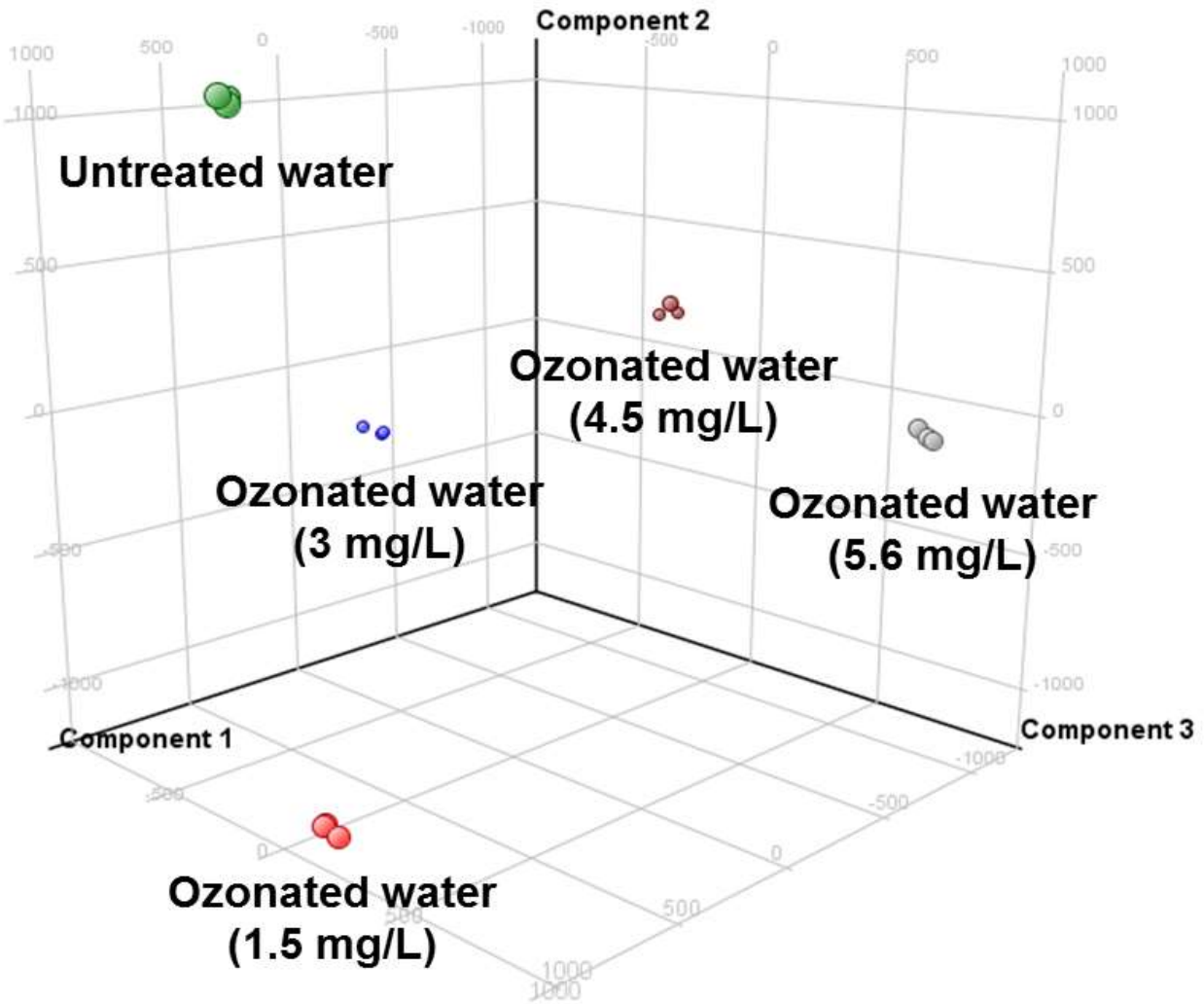
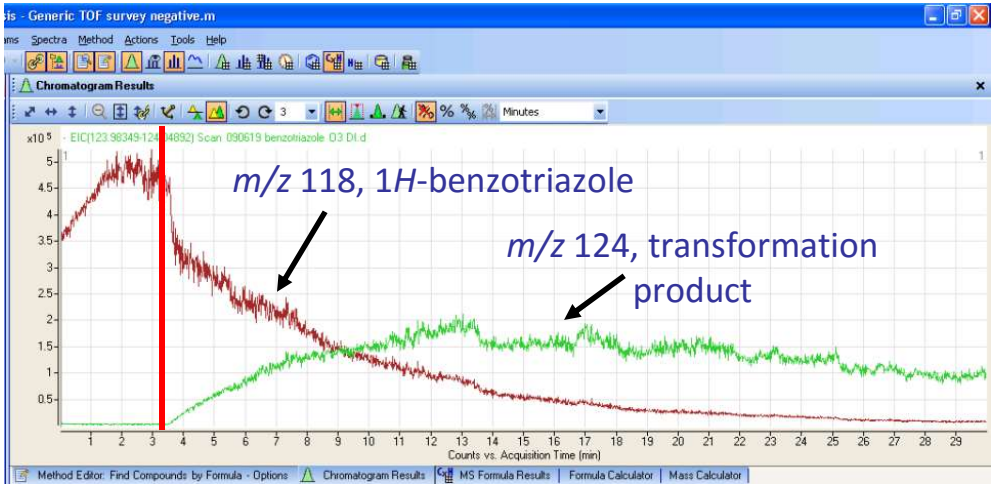
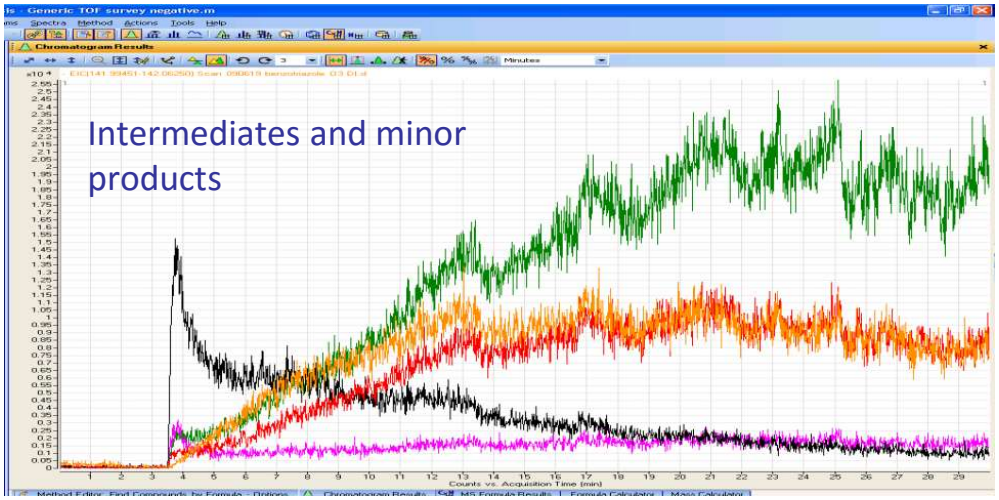
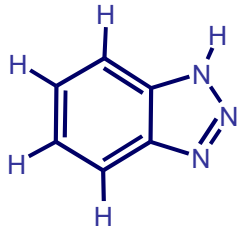


Merel S, Anumol T, Park M, Snyder SA. 2015 *J. Hazard. Mater.* 282:75-85

Benzotriazole Transformation

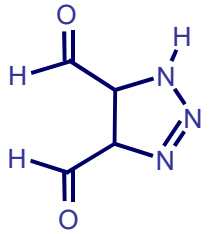






1*H*-benzotriazole

O_3/ ·OH



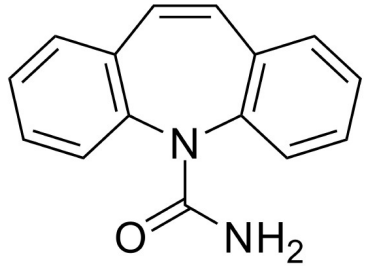
***m/z* 124.01522 (meas.)**
***m/z* 124.01525 (calc.)**
$C_4H_3N_3O_2$

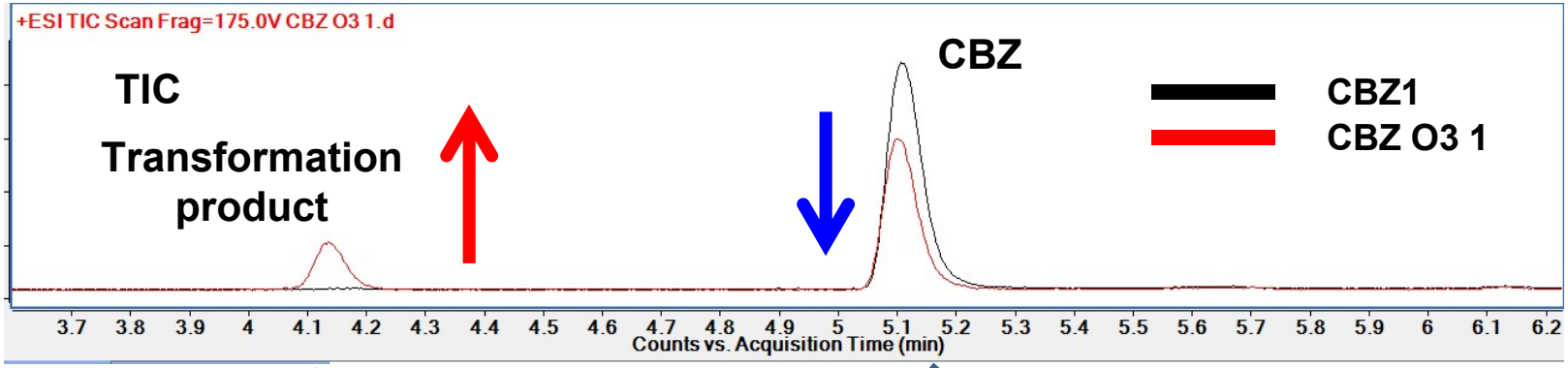
1,2,3-triazole-4,5-dicarbaldehyde

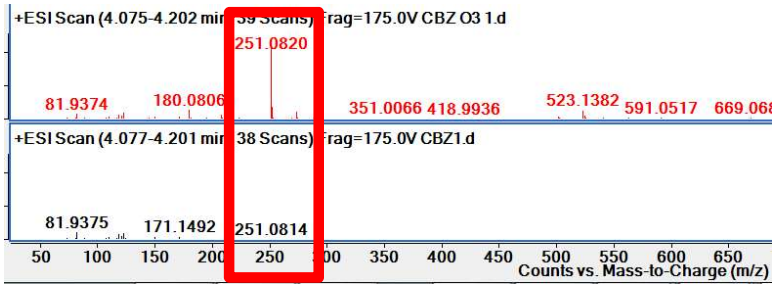
Mawhinney, DB, BJ Vanderford, SA Snyder. (2012) ***ES&T*** 46(13):7102-7111.

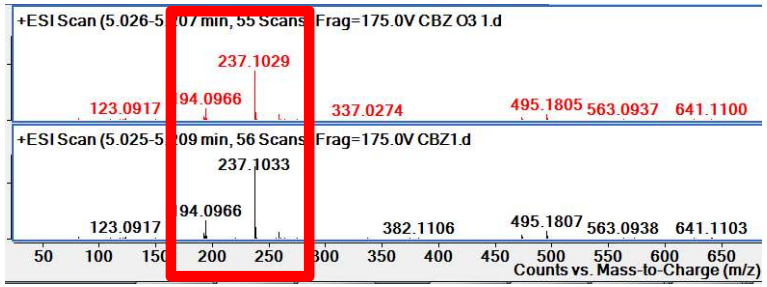
Carbamazepine Transformation

- CBZ (RT=5.1min) was oxidized by ozone





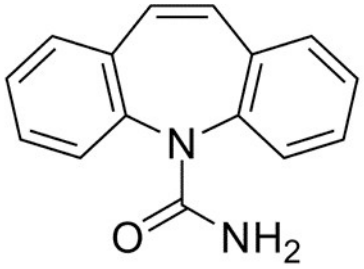




MS/MS Identifies CBZ Product

CBZ

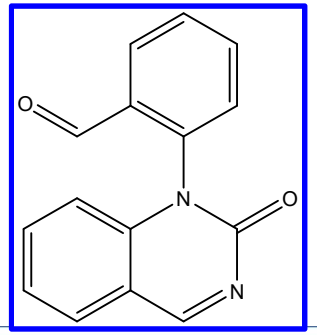
Carbamazepine

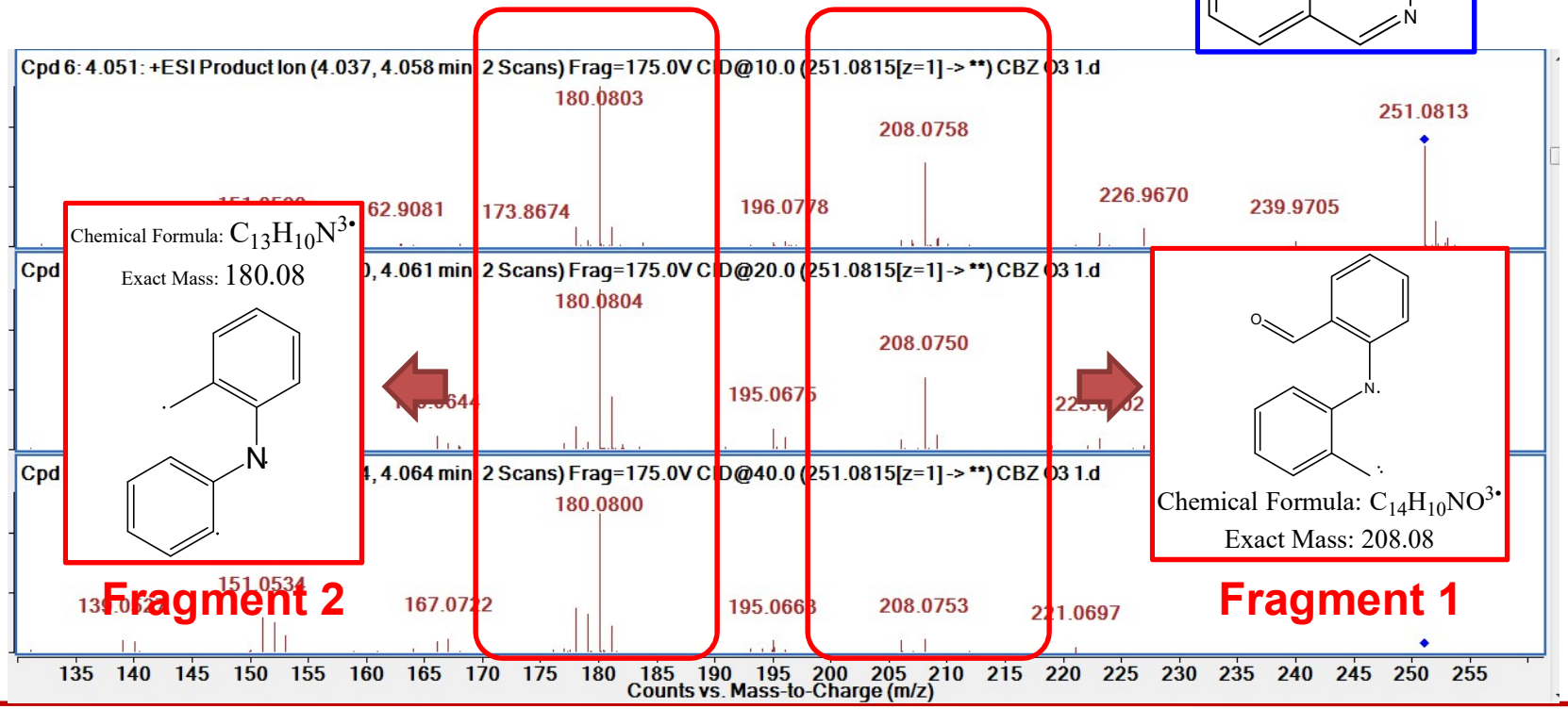


Ozone

BQM

1-(2-benzaldhyde)-4-hydro-(1H,3H)-quinazoline-2-one





NON-TARGETED EXAMPLE:

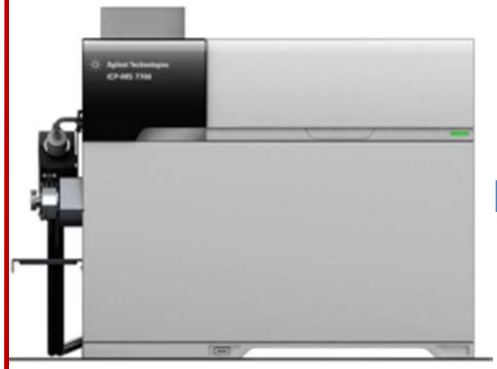
TRANSFORMATION PRODUCTS OF CHLORINE

GC-ICP-MS

Advantages:

- **No water present → little/no oxide interferences**
- **No evaporative cooling of plasma → lower RF forward power**
- **Lower RF power → fewer Ar-based ions ($^{40}Ar^+$, $^{38}Ar^{40}Ar^+$, $^{38}Ar^{40}ArH^+$, etc.)**

∴ **Interference-free analysis without collision gas**

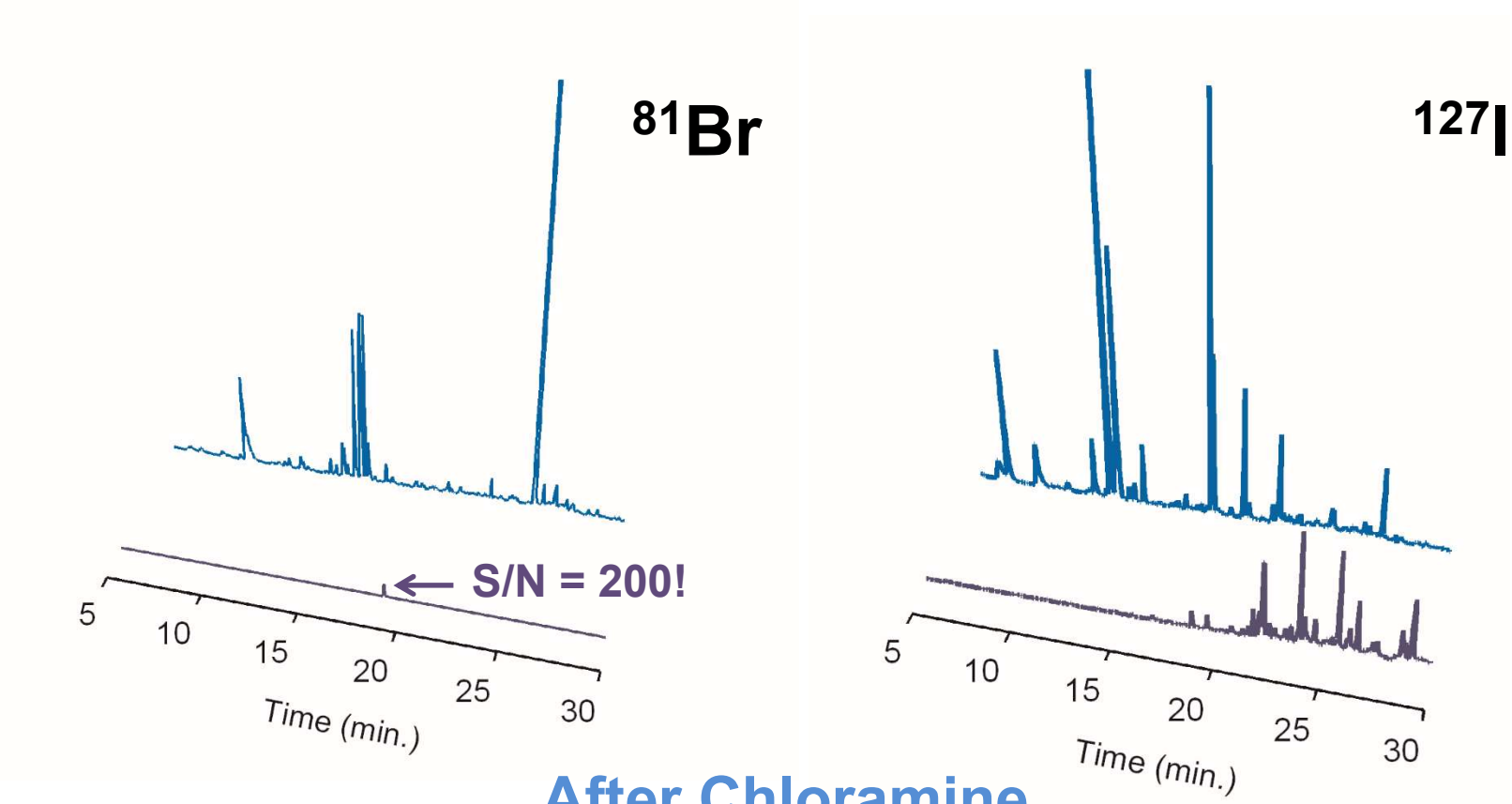


Model 7900 ICP-MS

Model 7890B GC

GC-ICP-MS

GC-ICP/MS: DBP Formation

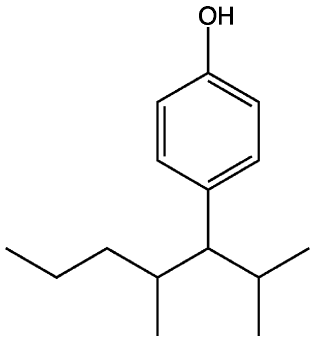


After Chloramine

Before Chloramine

Nonylphenol Transformation

- Used in antioxidants, oils, detergents, emulsifiers
- Technical mixture of many isomers
- Known to have estrogenic activity
- EPA Drinking Water CCL4 Draft List



1999 *Environ. Sci. Tech. 33(16) 2814-2829*

Analytical Methods for Detection of Selected Estrogenic Compounds in Aqueous Mixtures

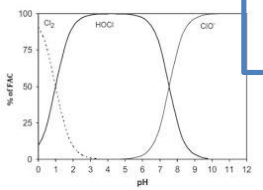
SHANE A. SNYDER,*,† TIMOTHY L. KEITH,† DAVID A. VERBRUGGE,† ERIN M. SNYDER,† TIMOTHY S. GROSS,‡ KURUNTHACHALAM KANNAN,† AND JOHN P. GIESY†

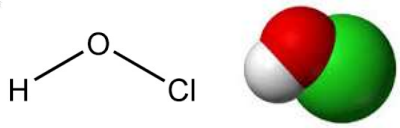
can operate through a number of both direct and indirect mechanisms of action, of particular concern are those compounds that mimic endogenous estrogens. The Safe Drinking Water Act Amendments of 1995 (Bill No. S.1316) and the Food Quality Protection Act of 1996 (Bill No. P.L. 104-170), which mandate comprehensive screening for estrogenic and anti-estrogenic chemicals, are examples of the increasing public concern regarding endocrine disruption. While it is known that many natural and synthetic chemicals are estrogenic, it is unclear whether the concentrations of estrogenic agents present in the environment are sufficient to cause adverse physiological effects. One aspect of conducting human or wildlife risk assessments is an

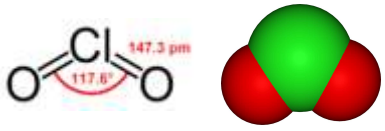
Nonylphenol Oxidation

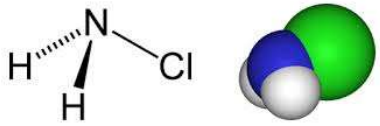
0.04M Phosphate buffer,
1ppm Nonylphenol
6.3uM Bromide, 6.3uM Iodide

4ppm Chlorine	4ppm Chlorine Dioxide	4ppm Monochloramine
10min	10min	10min
30min	30min	30min
1h	1h	1h
2h	2h	2h
5h	5h	5h









Analytical Strategy

Samples

- **LLE w/ Hexane**
  - **GC-QTOF**
  - **GC-ICP-MS**
- **SPE**
  - **LC-QTOF**
  - **LC-QQQ**
  - **LC-ICP-MS**
  - **GC-QTOF**
  - **Bioassay**
- **Direct injection**
  - **IC-ICP-MS**

Volatiles and semi-volatiles unknowns

Agilent 7200 GC-Q-TOF

Polar unknowns

Agilent 6540 LC-Q-TOF

Halogenated organics

Agilent 7900 ICP-MS

Oxahalides (BrO_3^-; IO_3^-) and Ions (Cl^-, Br^-, I^-)

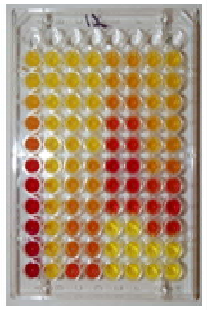
Metrohm 850 IC+ Agilent 7900 ICP-MS

Halogenated volatiles and semi-volatiles

Agilent 7900 ICP-MS

Targeted analysis of identified DBPs

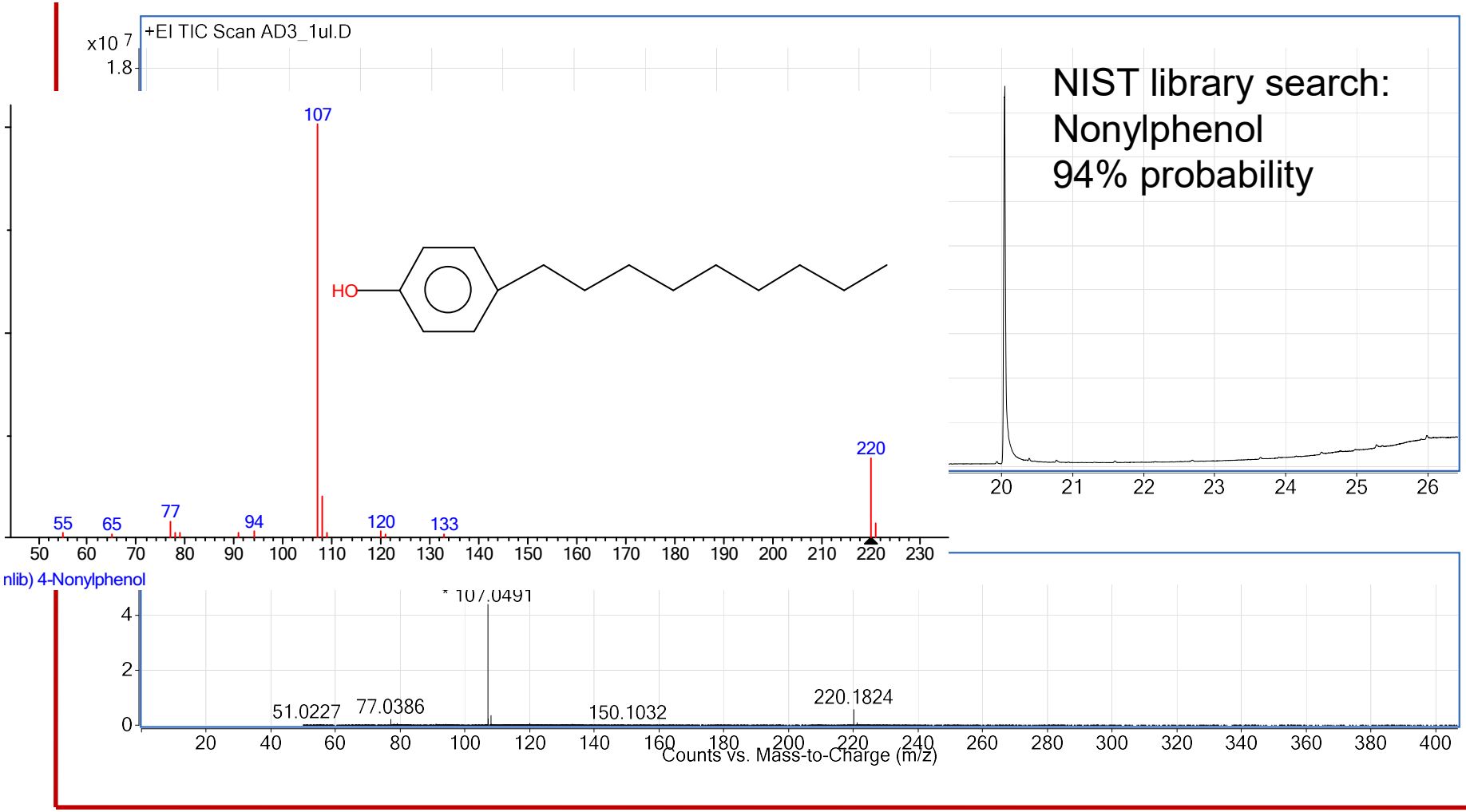
Agilent 6490 LC-MS/MS



Mutagenicity
Genotoxicity
p53 induction
Cytotoxicity
Oxidative stress
Estrogenic effects
Glucocorticoid effects …

Bioassays

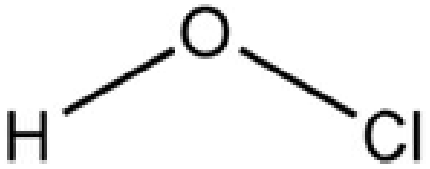
Nonylphenol – GC-QTOF

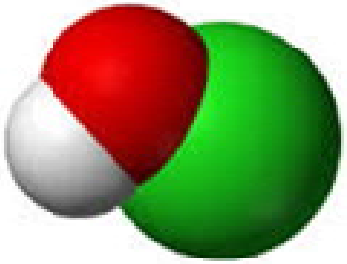


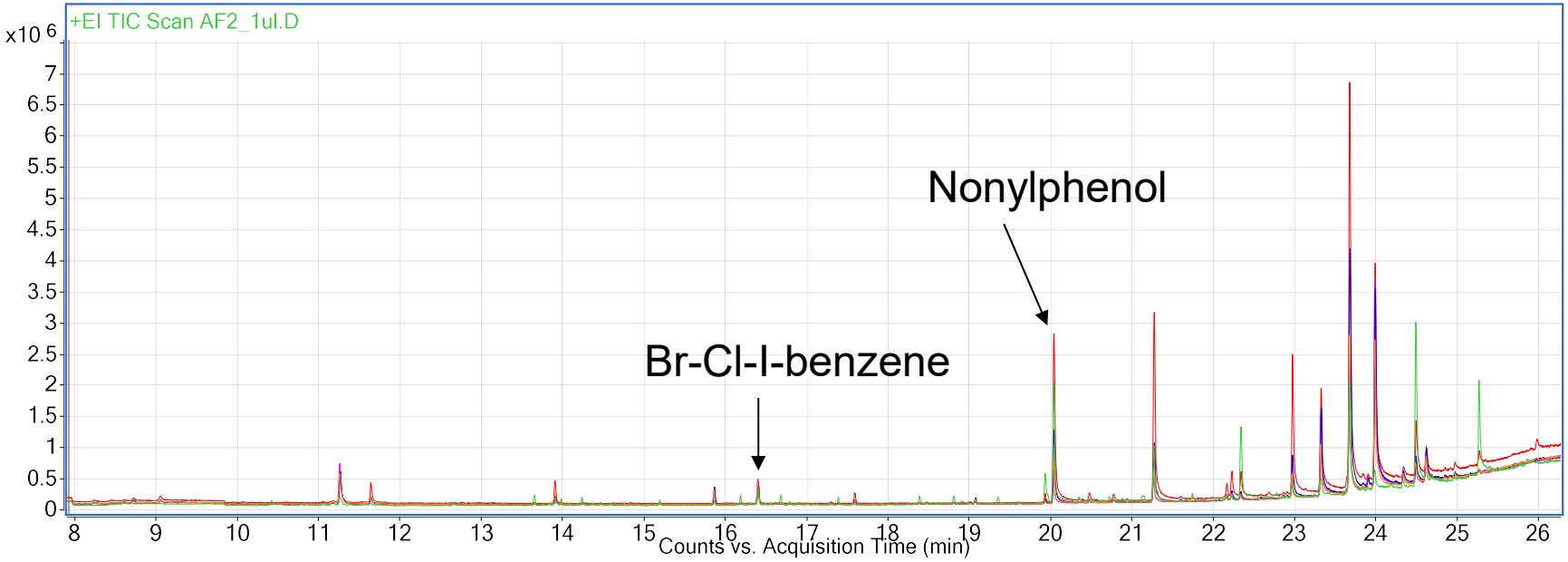
NP Oxidation with Chlorine

DBP formation after oxidation with chlorine:

- 10min
- 30min
- 1h
- 2h
- 5h



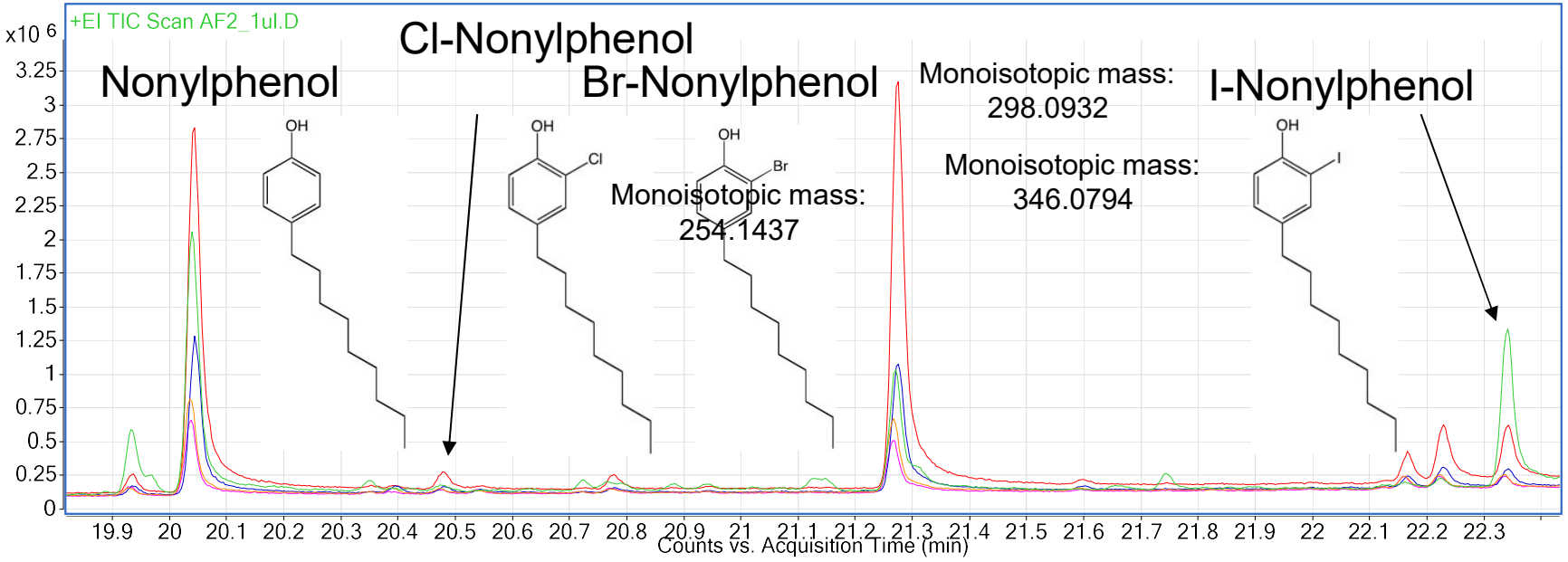


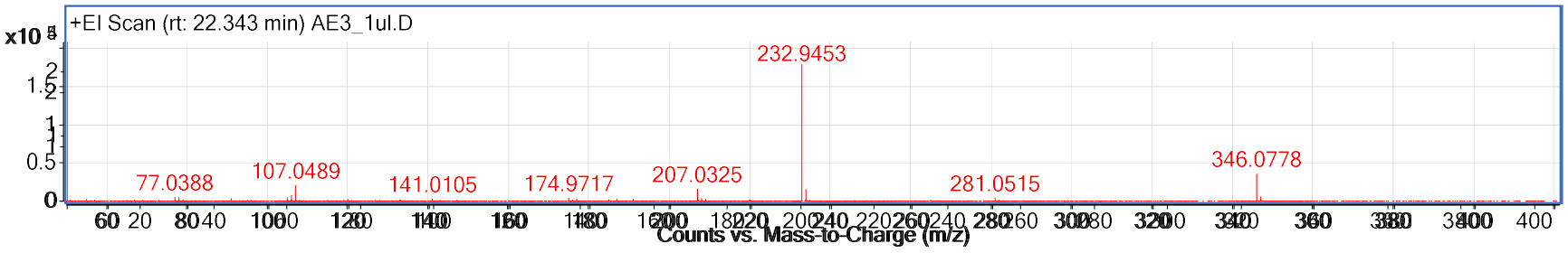


NP Oxidation with Chlorine

Monohalogenated DBPs: (19-22 min retention times)

10min 30min 1h 2h 5h

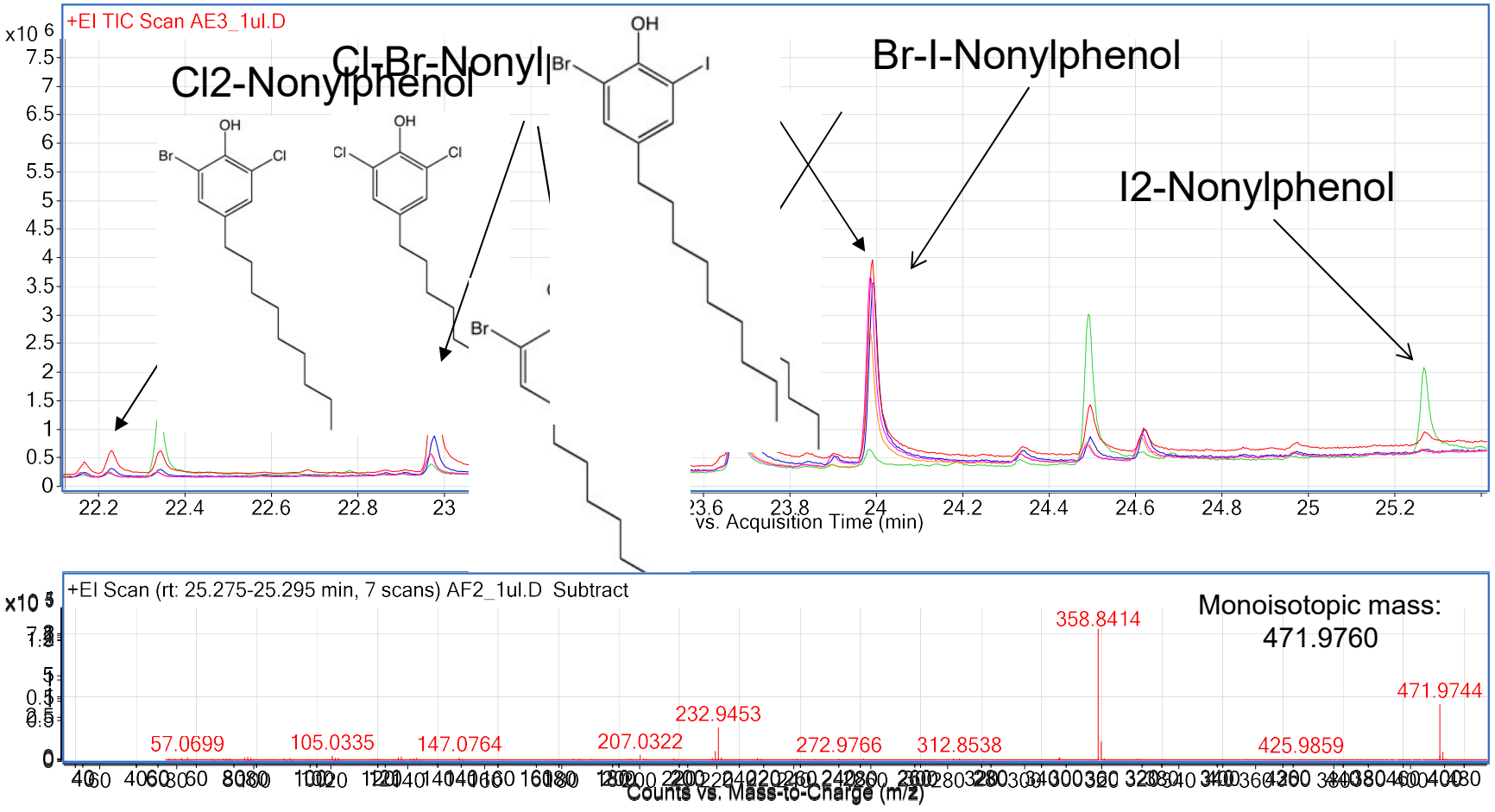




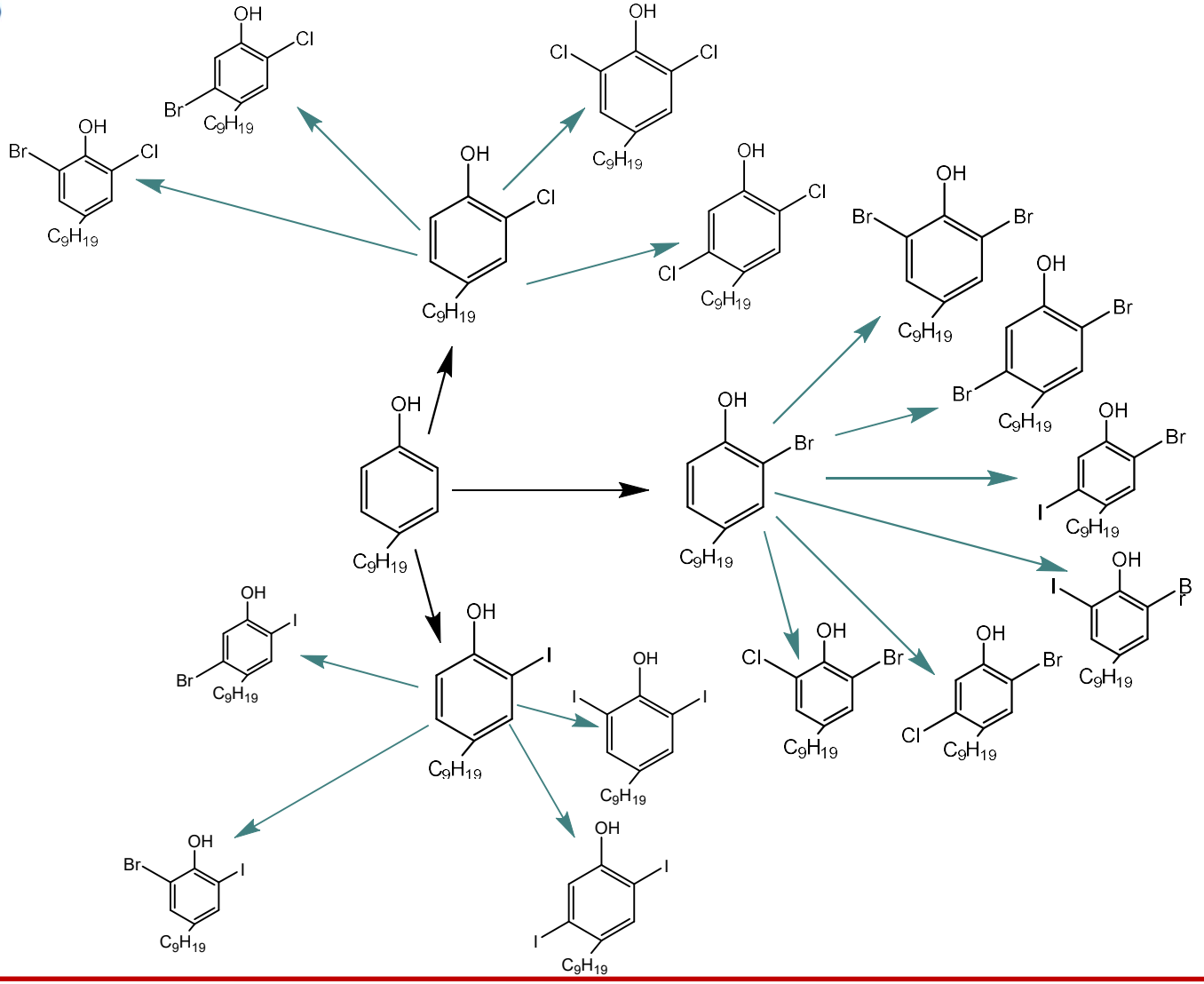
NP Oxidation with Chlorine

Dihalogenated DBPs (22-26 min retention times)

10min 30min 1h 2h 5h



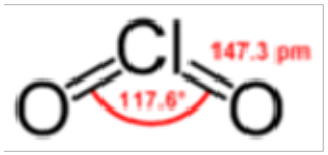
NP Transformation Pathway (OCl)

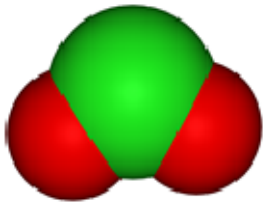


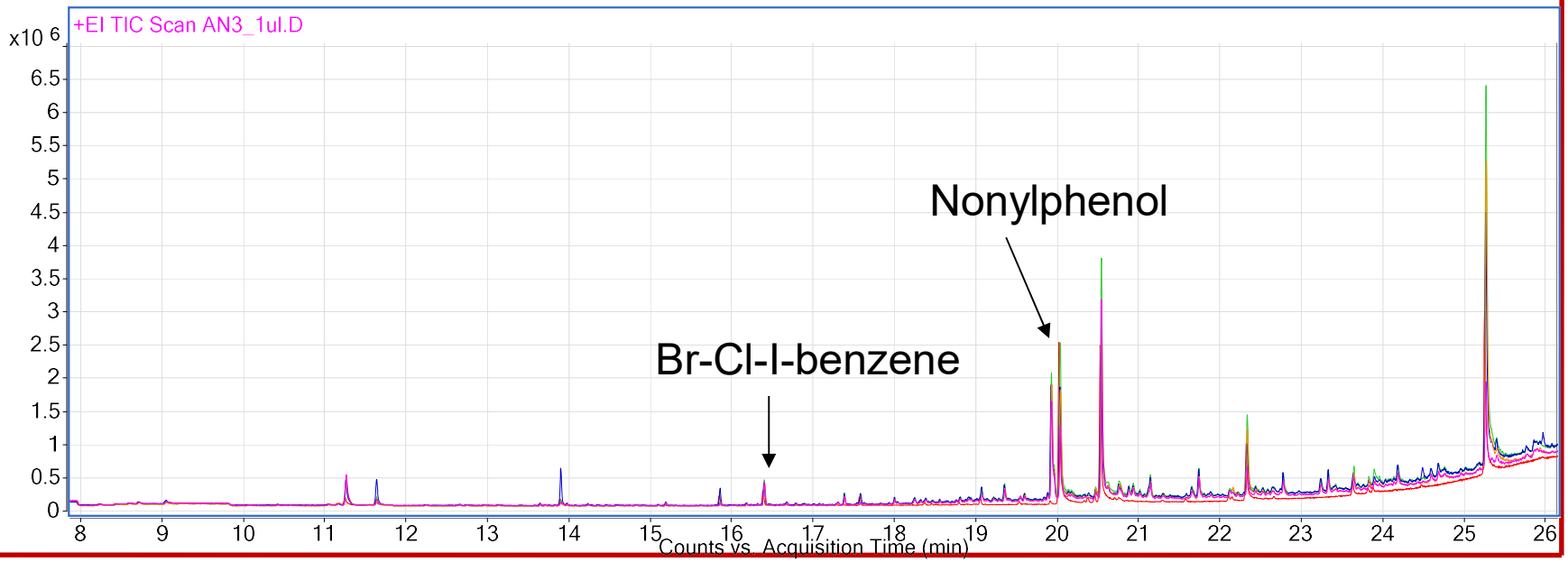
NP Oxidation w/Chlorine Dioxide

DBP formation after oxidation with chlorine dioxide:

- 10min
- 30min
- 1h
- 2h
- 5h





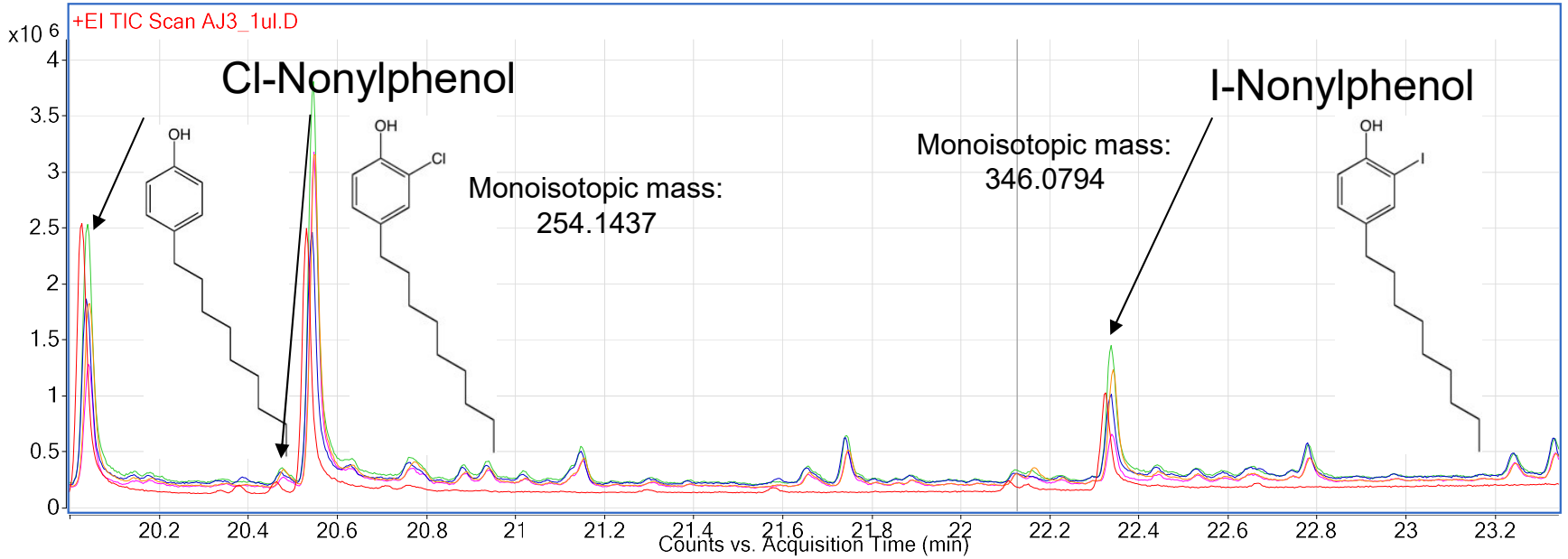


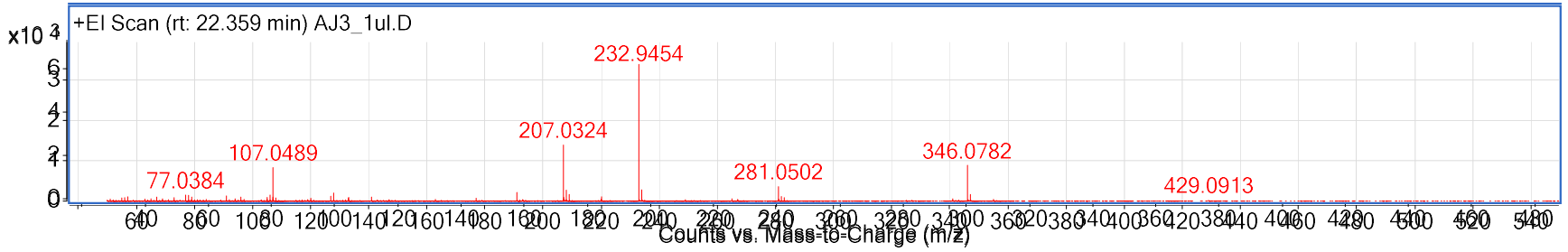
NP Oxidation w/Chlorine Dioxide

Monohalogenated DBPs:

Nonylphenol

10min 30min 1h 2h 5h

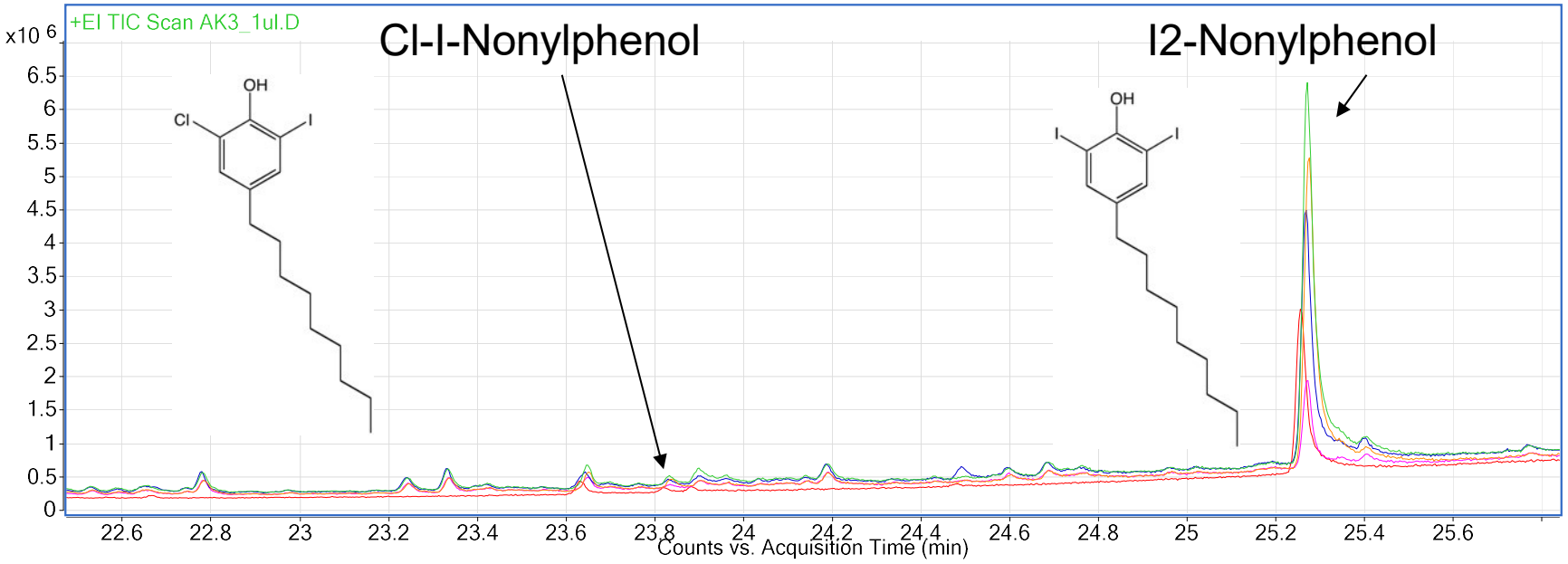


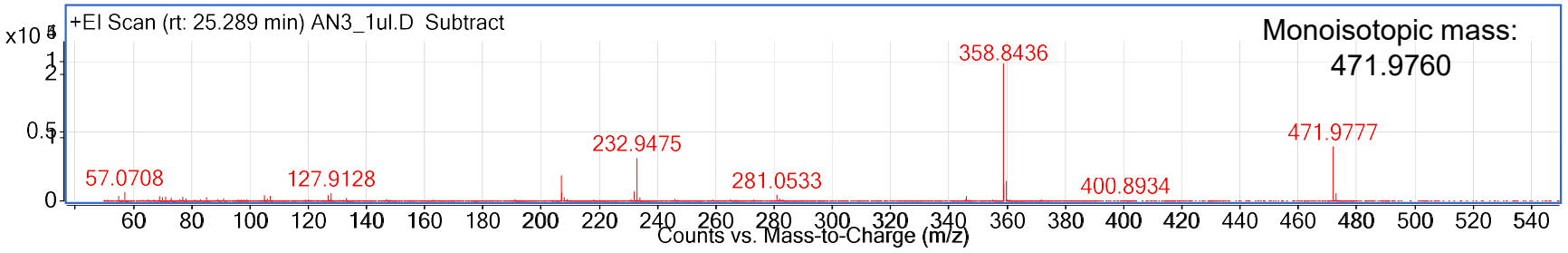


NP Oxidation w/Chlorine Dioxide

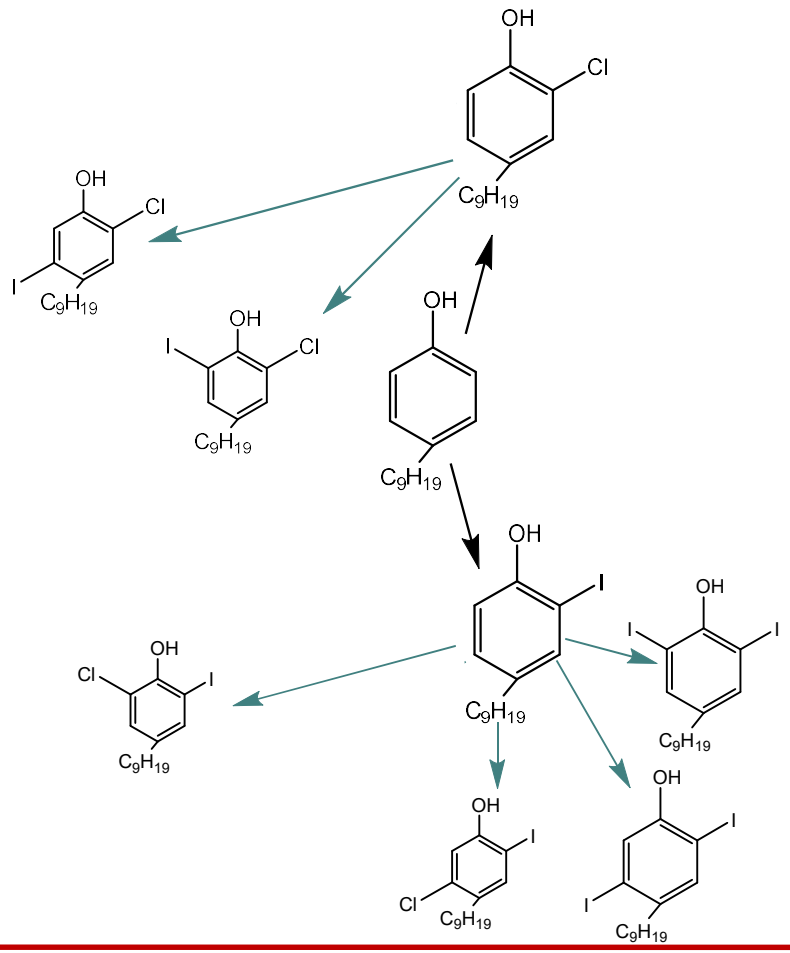
Dihalogenated DBPs:

10min 30min 1h 2h 5h





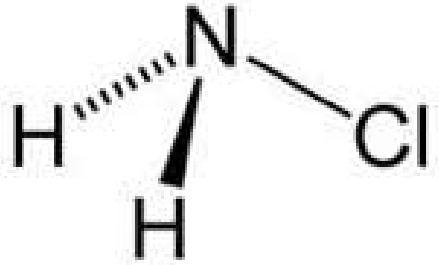
Transformation Pathway (ClO_2)

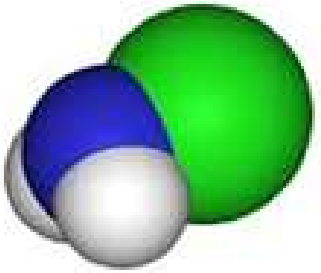


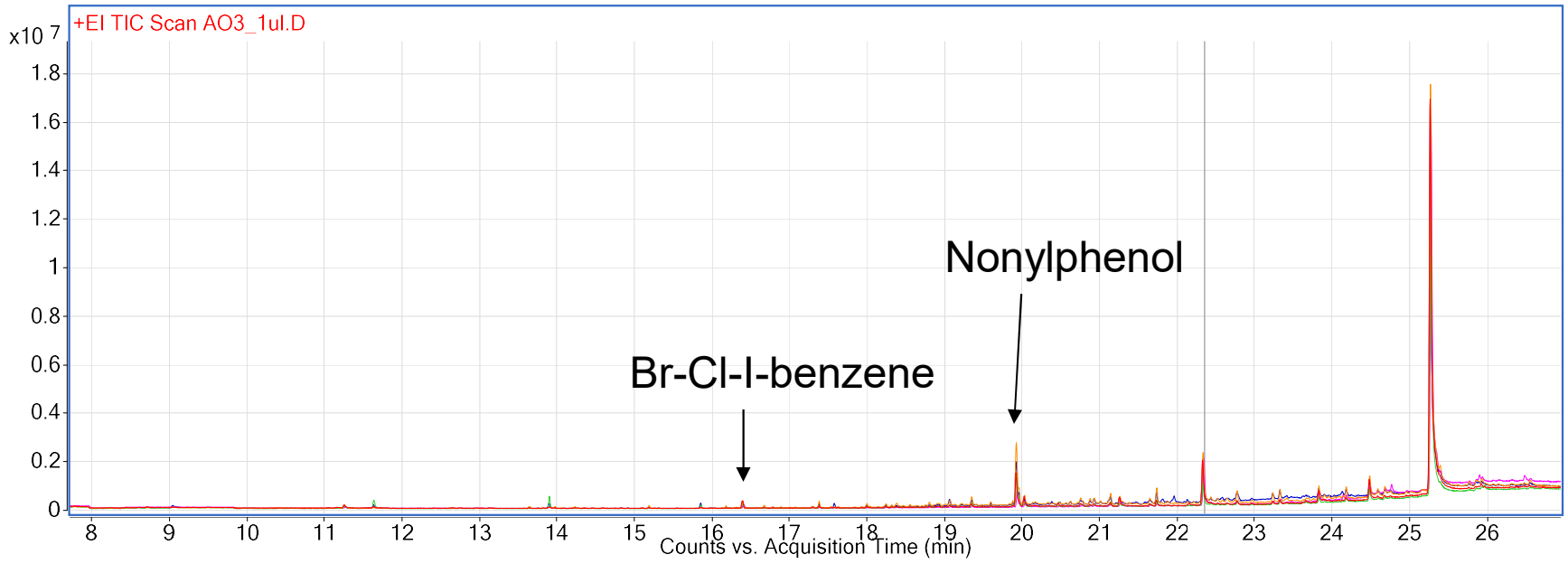
NP Oxidation with Monochloramine

DBP formation after oxidation with monochloramine:

- 10min
- 30min
- 1h
- 2h
- 5h



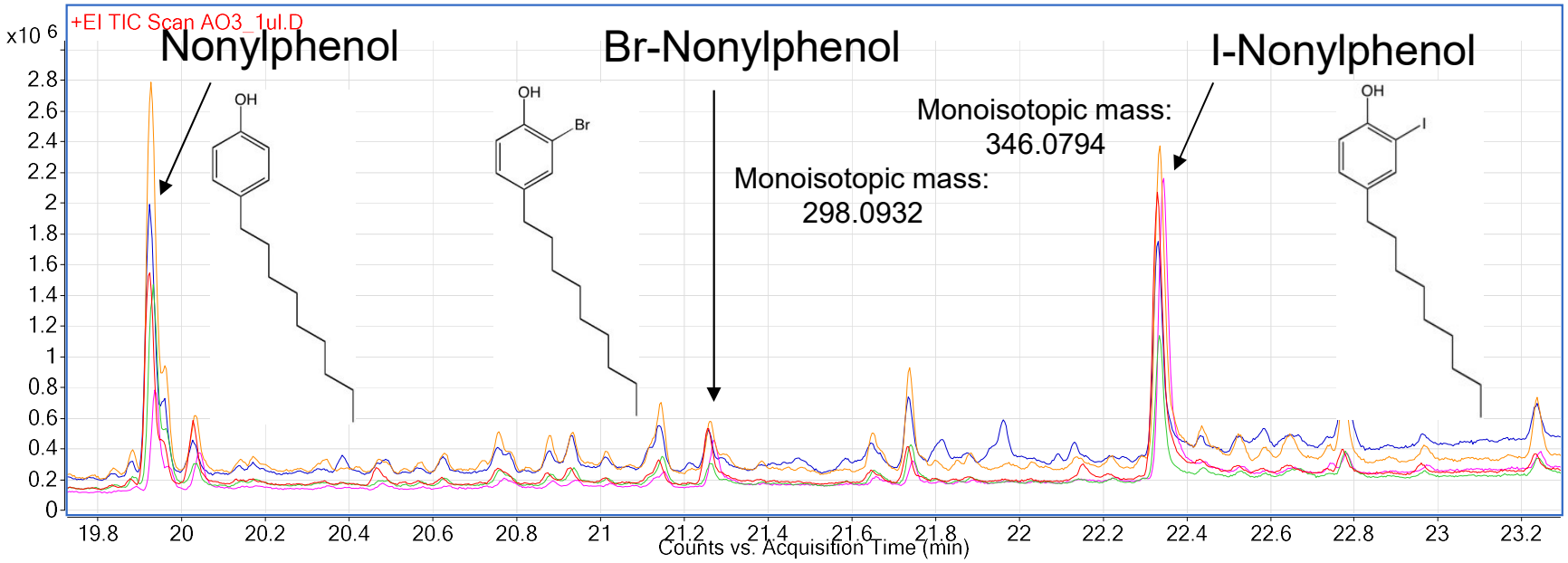




NP Oxidation w/Chloramine

Monohalogenated DBPs:

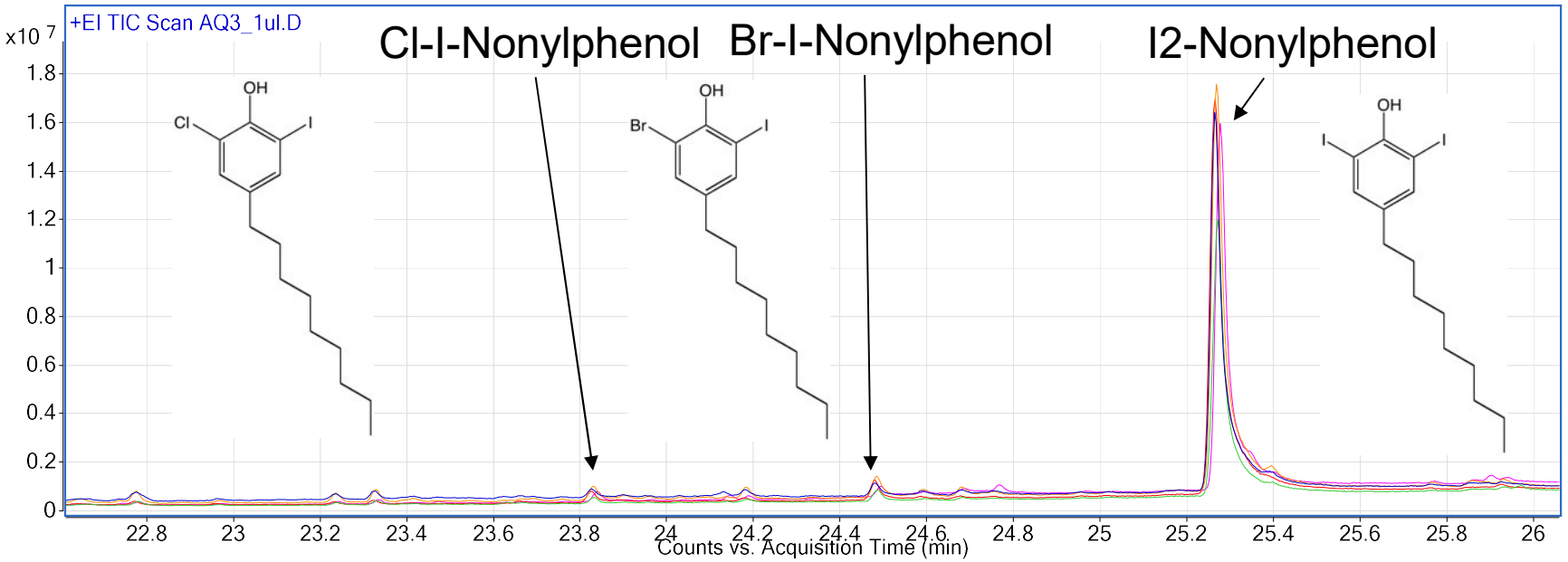
10min 30min 1h 2h 5h

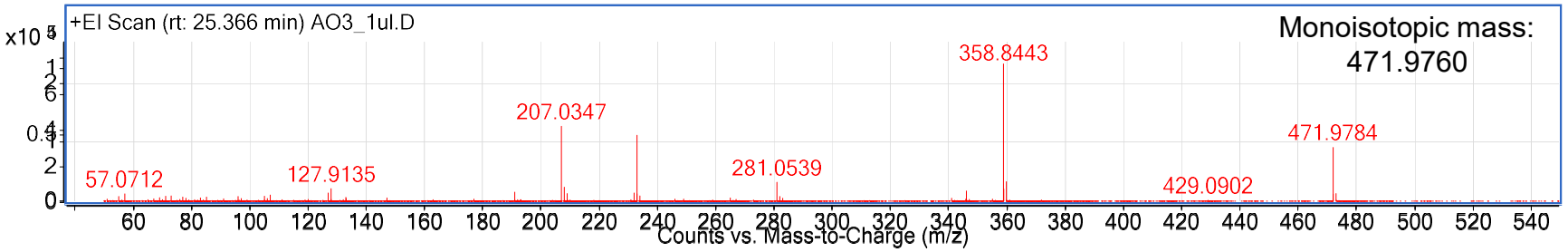


NP Oxidation w/Chloramine

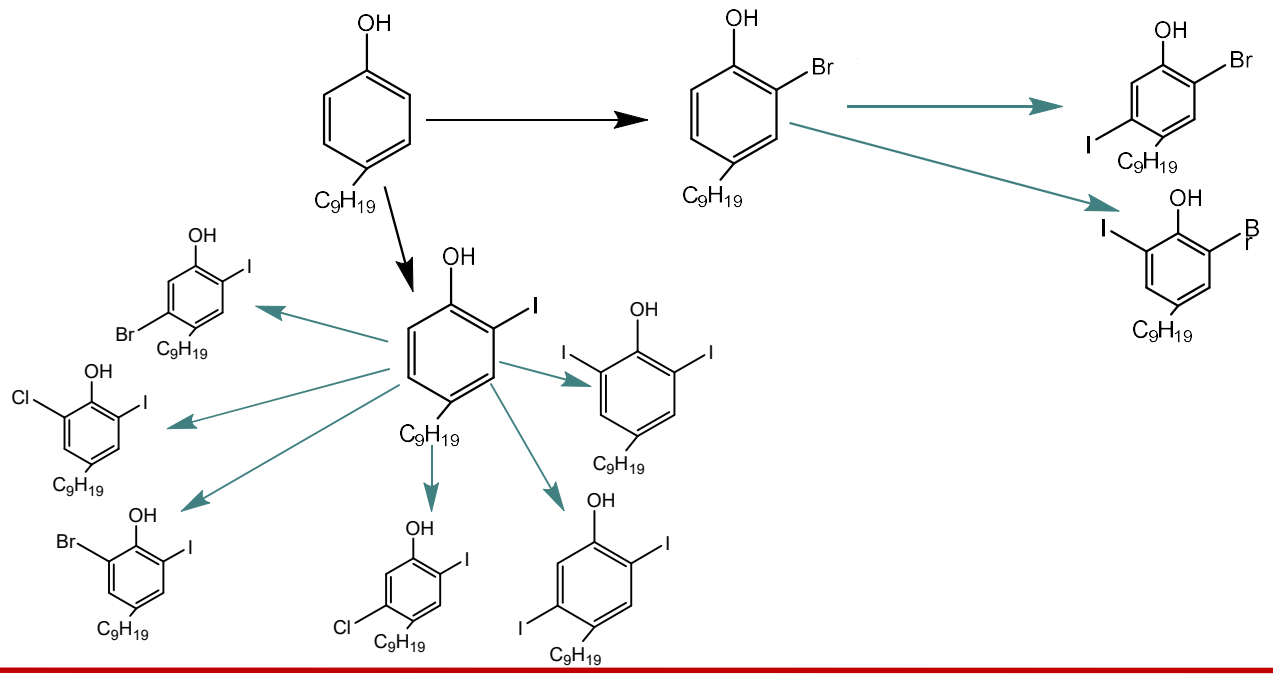
Dihalogenated DBPs:

10min 30min 1h 2h 5h





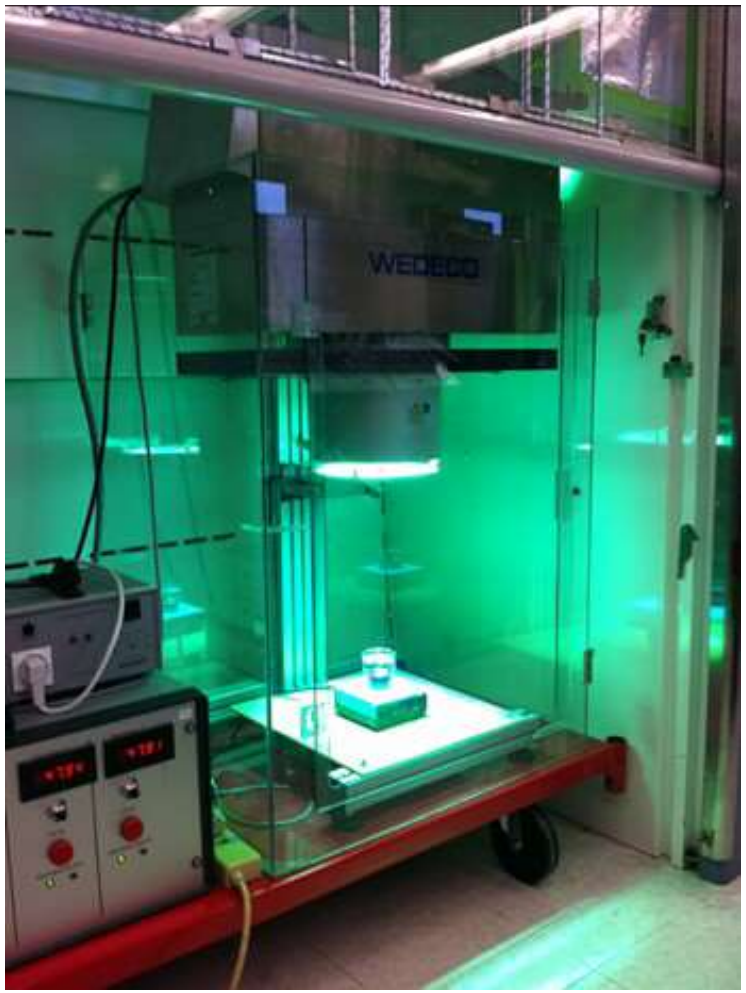
Transformation Pathway (NH2Cl)



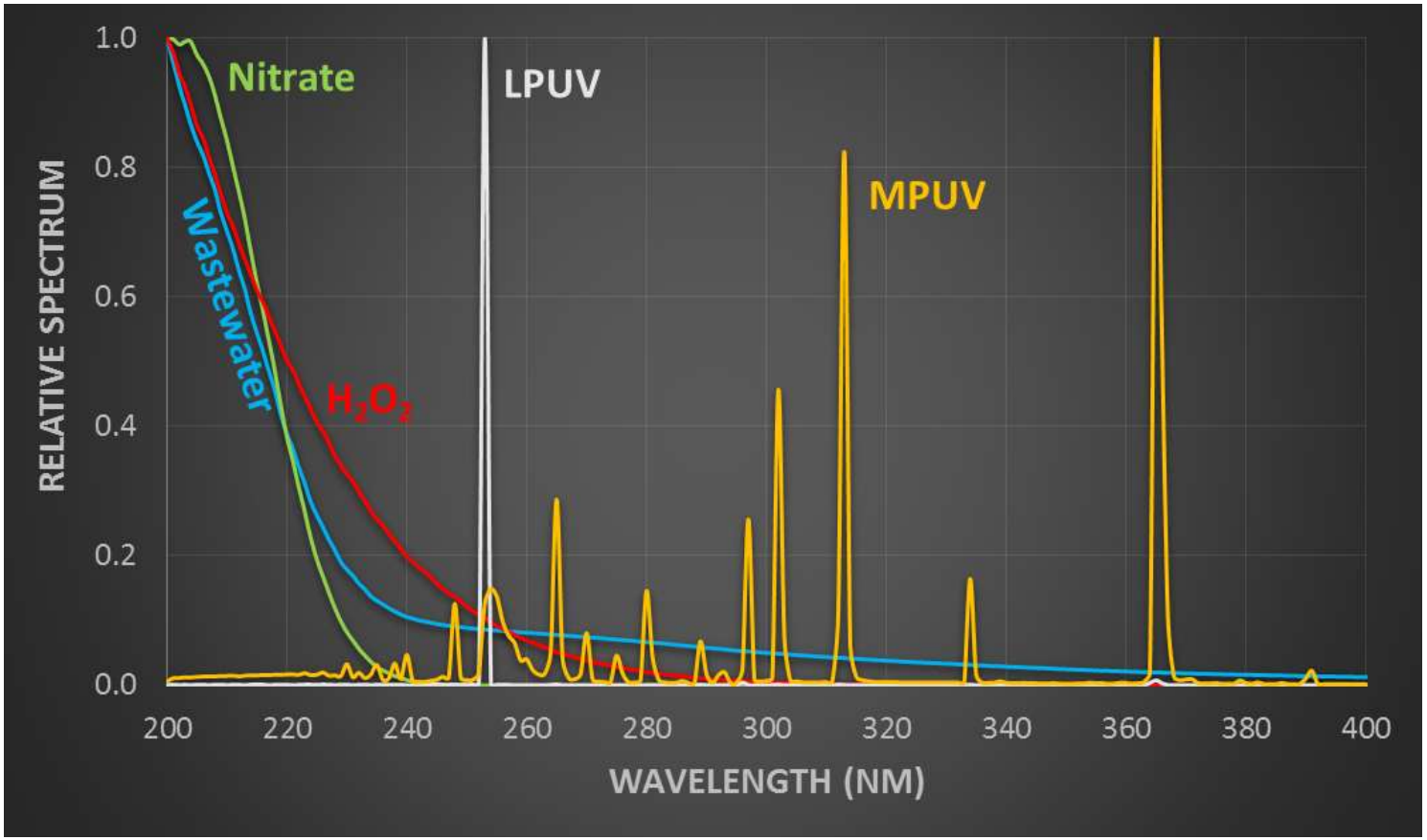
NON-TARGETED EXAMPLE:

TRANSFORMATION PRODUCTS OF UV AOP

UV Transformation Products



Formation of nitrogenous byproducts through UV photolysis



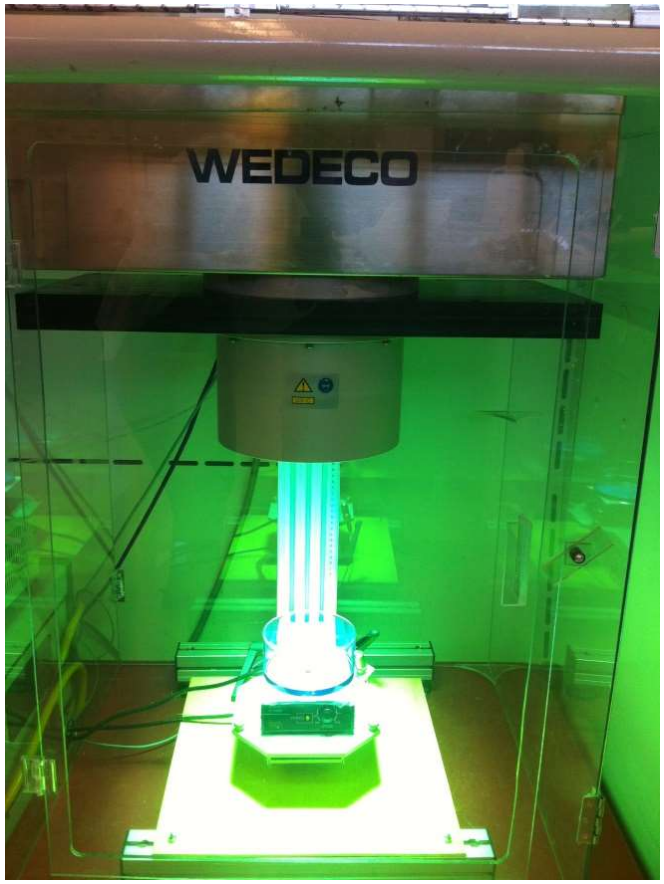
- Formation of nitrogenous byproducts (nitro- or nitroso aromatic compounds) through nitrate photolysis with organic precursors

Experimental matrix for UV AOP genotoxicity

- UV lamp type: low pressure (LP) and medium pressure (MP)
- Test water: Secondary treated wastewater (Ina Rd. WWTP)
- Oxidant: Hydrogen peroxide

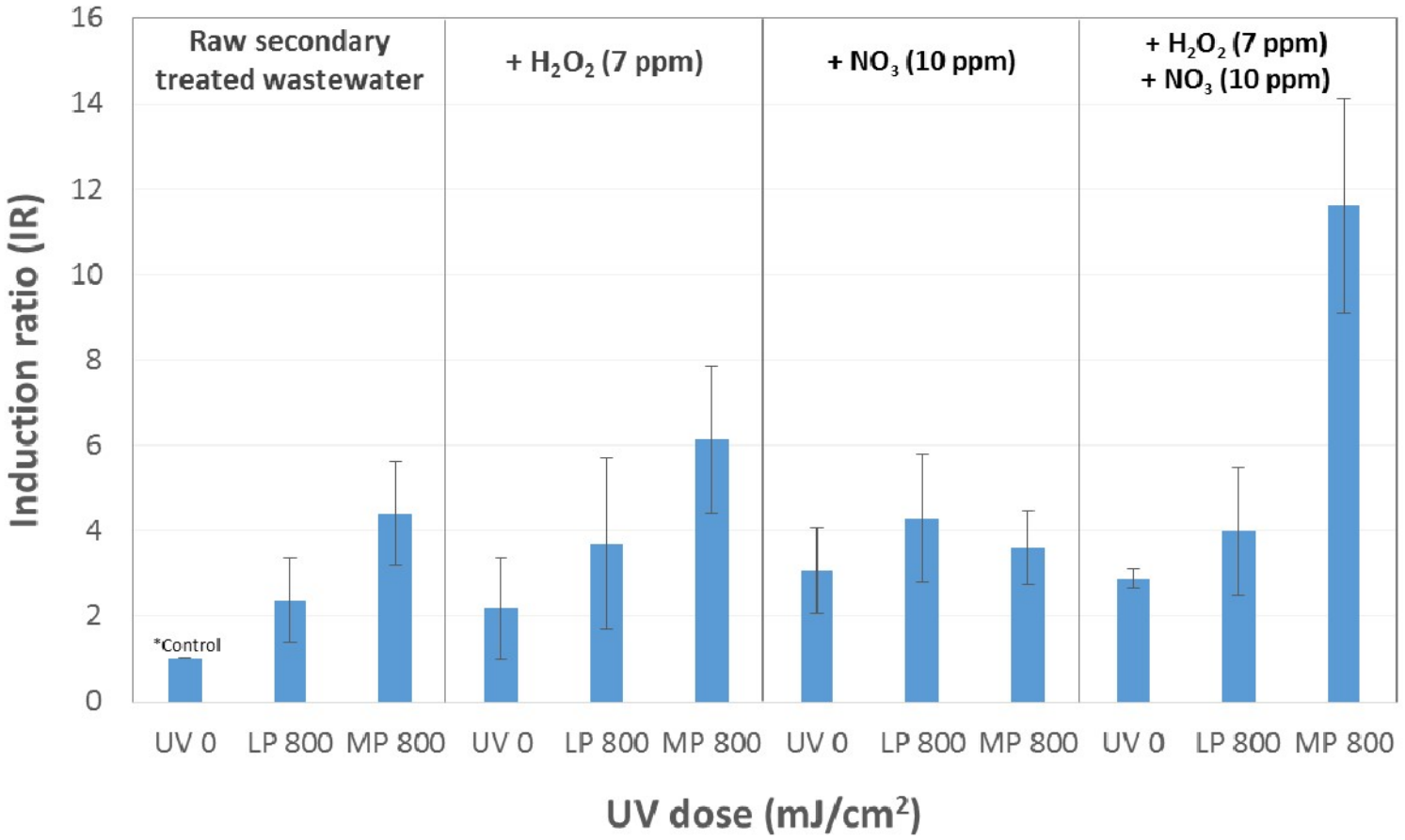
		Nitrate (0 mg/L)		Nitrate (10 mg/L)	
LP·MP UV /H_2O_2 AOP		H_2O_2 (mg/L)		H_2O_2 (mg/L)	
		0	**7**	**0**	**7**
UV dose (mJ/cm^2)	**0**	*X*	*X*	*X*	*X*
	400	*X*	*X*	*X*	*X*
	800	*X*	*X*	*X*	*X*

UV collimated beam device



Ames mutagenicity test

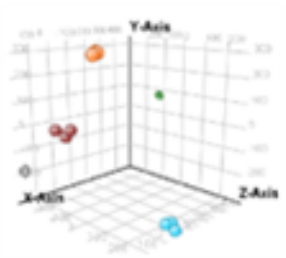
- Mutagenicity strain: *Salmonella typhimurium (*TA98 w/o S9)
- Type of reversion mutation: Frameshift



Identification of Genotoxicant

Characterization of a larger set of unknown compounds

Sampl e → LC/MS of sample and overlayed extracted chromatograms → Data alignment and analysis → Validation with MS/MS from standards



↓

Compound profile in a sample

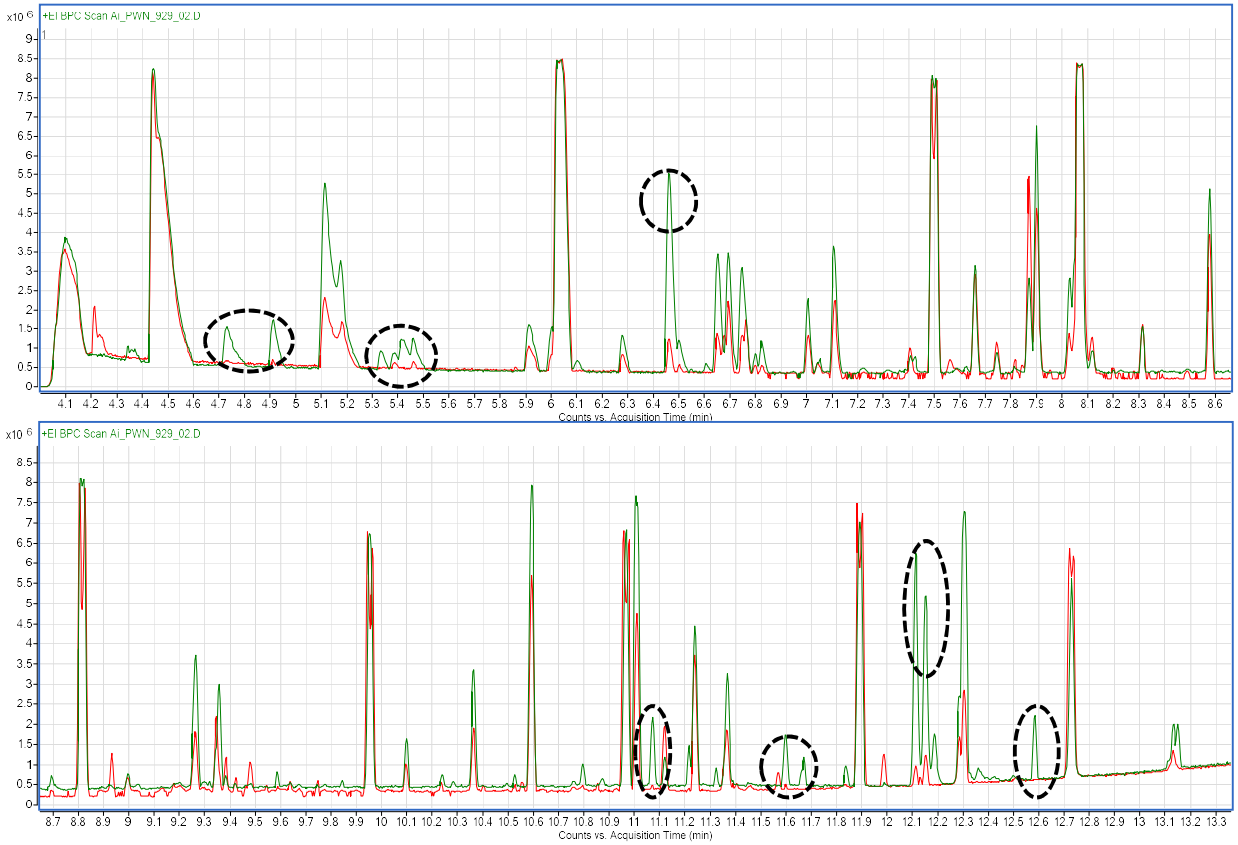
LC/MS QTOF

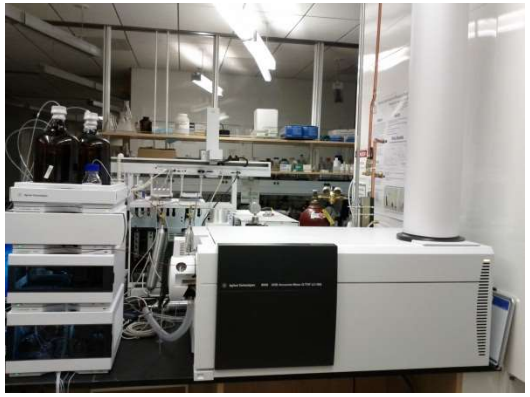
Polar or moderately polar compounds

GC/MS QTOF

Volatile and semi-volatile compounds

Discovery of New DBPs





LC-QTOF

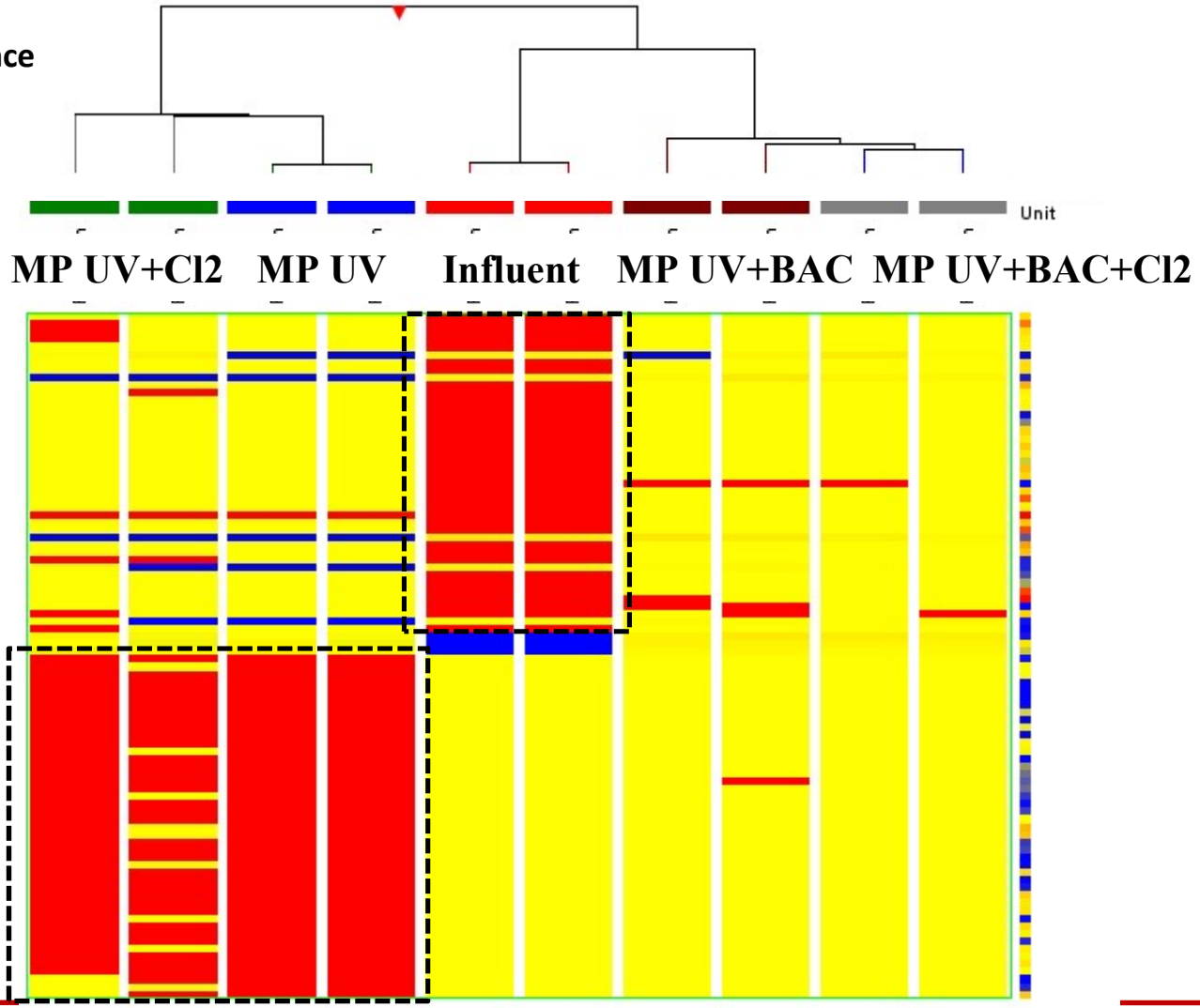
GC-QTOF

Red = Before MP UV

Green = After MP UV

Discovery of Novel UV Transformation Products

Color by normalized abundance



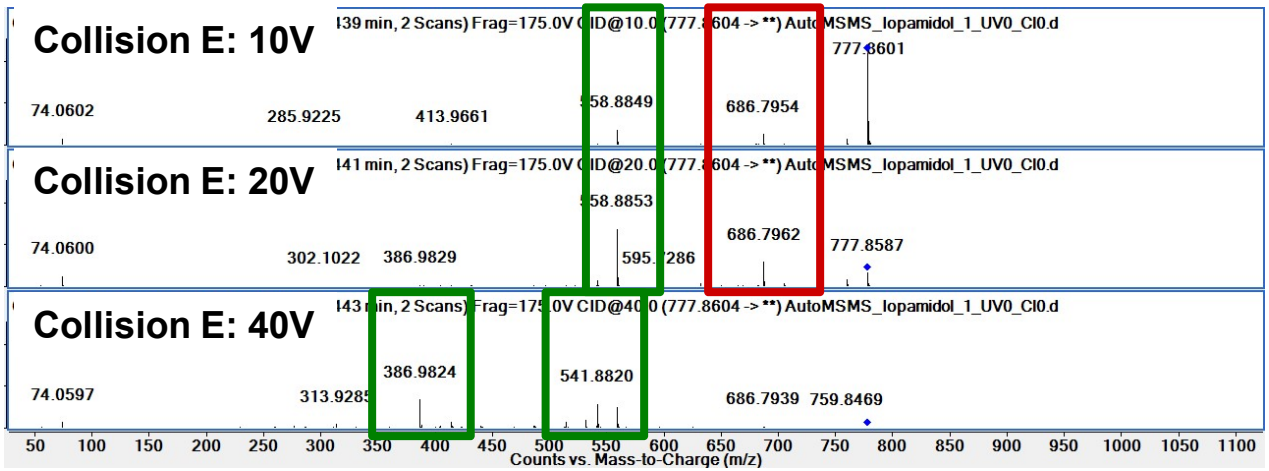
UV Attenuated !

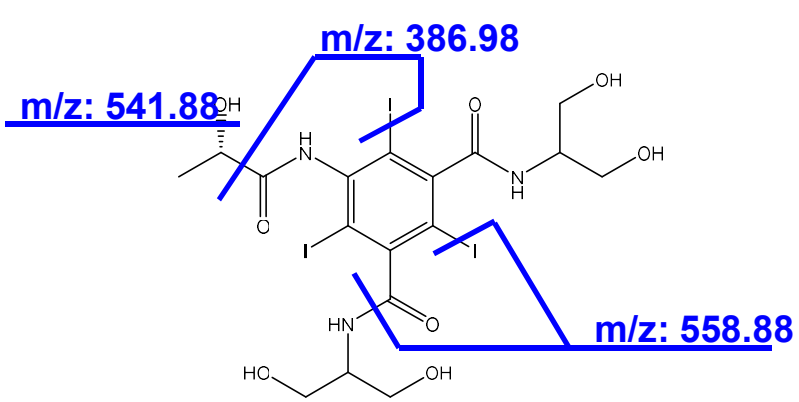
New By-products ?

Software: Agilent Mass Profiler Professional (MPP)

Fragmentation pattern of lopamidol

MS/MS spectra of lopamidol





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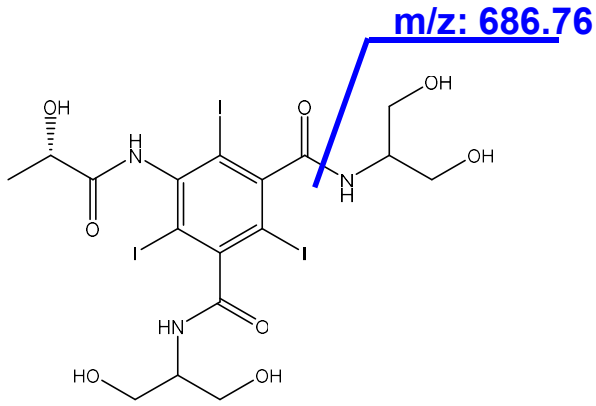

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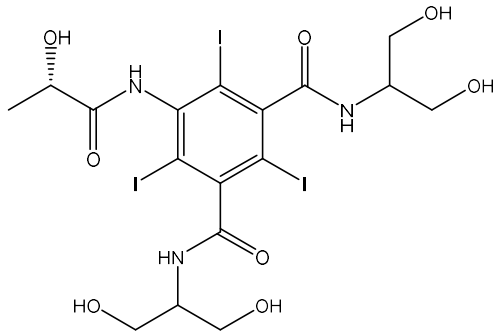
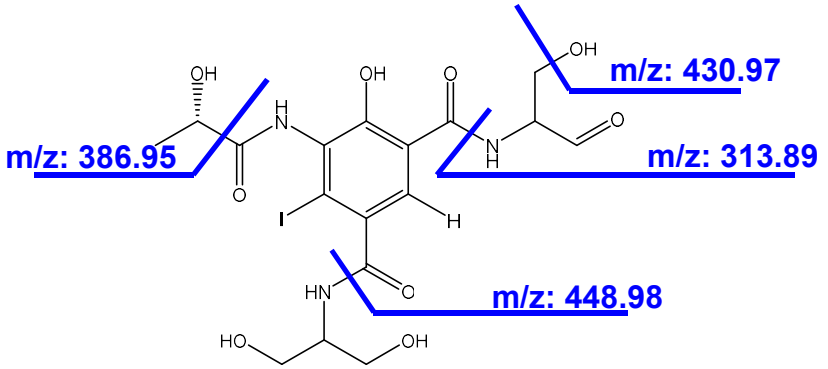
Transformation product of Iopamidol

$C_{17}H_{22}INO_9$ (Proposed structure)

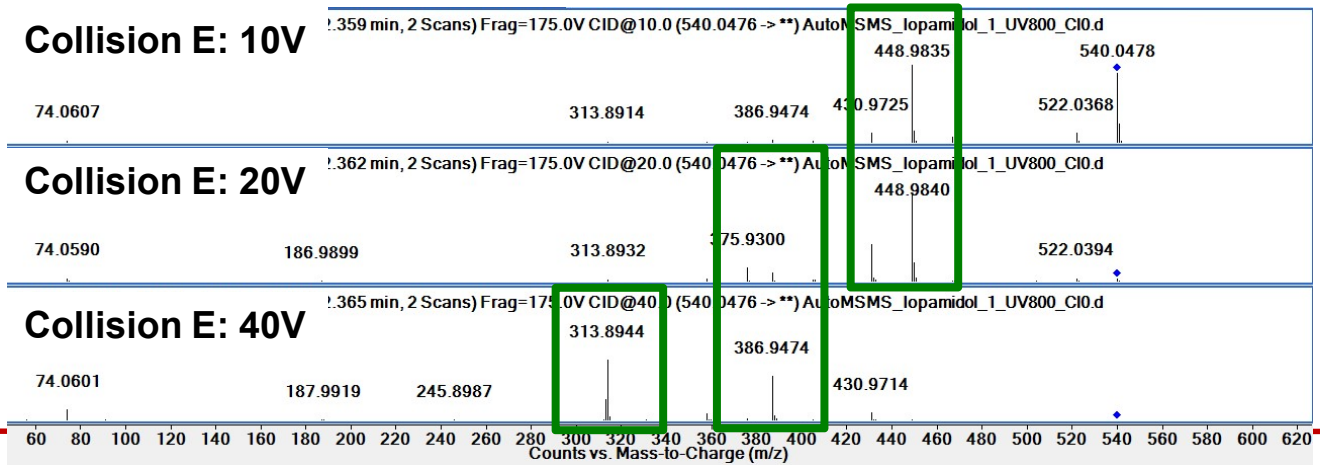


UV



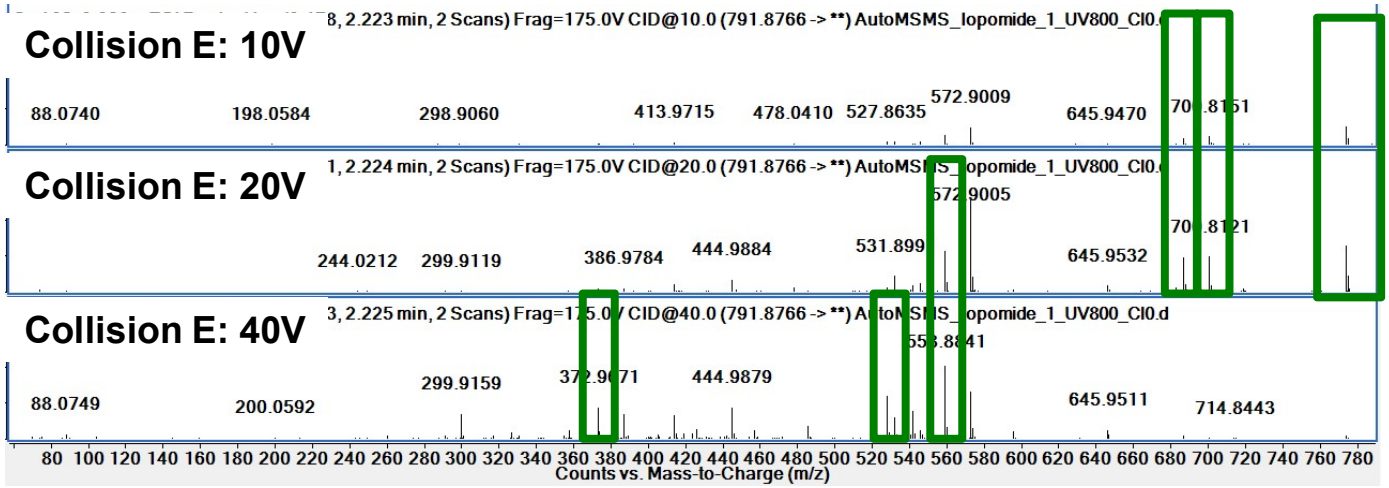
$C_{17}H_{22}I_3N_3O_8$ (Iopamidol)

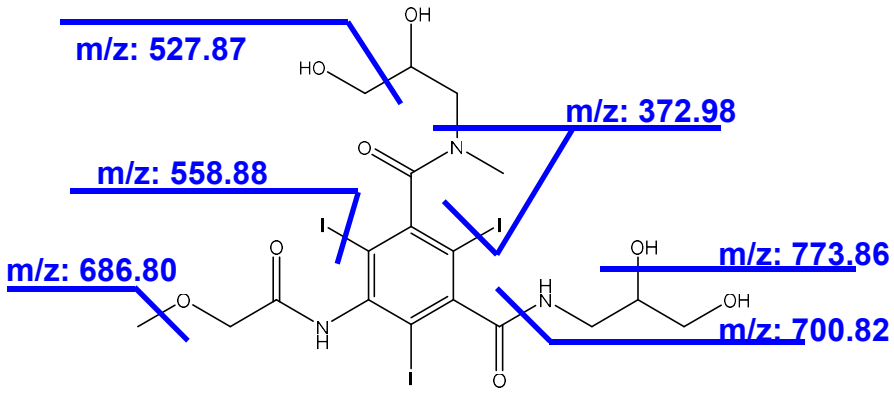
$C_{17}H_{22}INO_9$ (Proposed structure)



Fragmentation pattern of lopromide

MS/MS spectra of lopromide

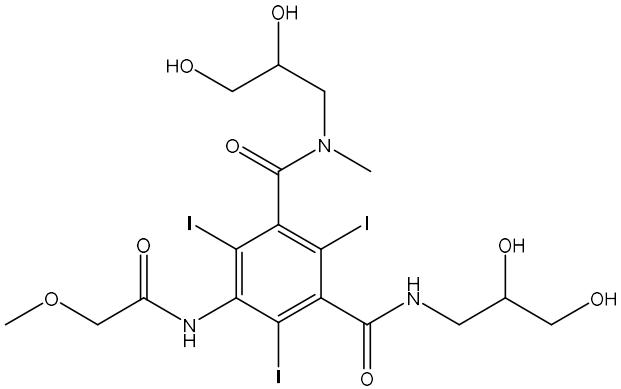




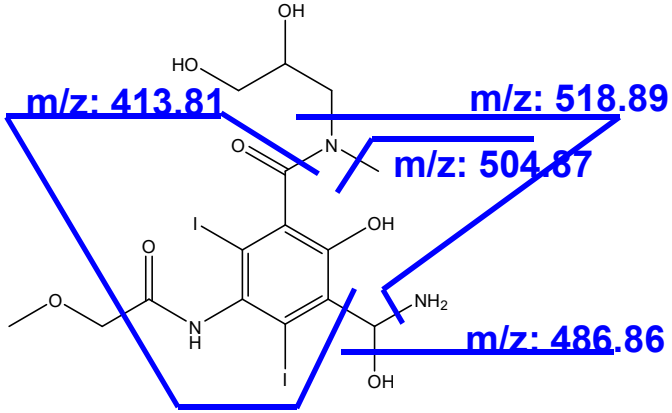
< Fragmentation pattern >

Transformation product of Iopromide

$C_{15}H_{21}I_2N_3O_7$ (Proposed structure)

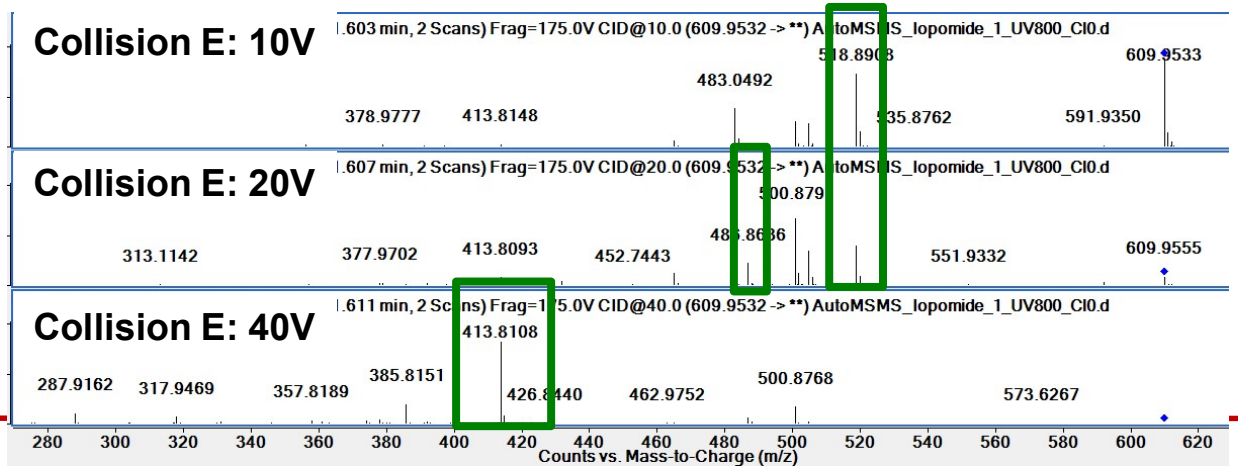


UV



$C_{18}H_{24}I_3N_3O_8$
(Iopromide)

$C_{15}H_{21}I_2N_3O_7$
(proposed structure)

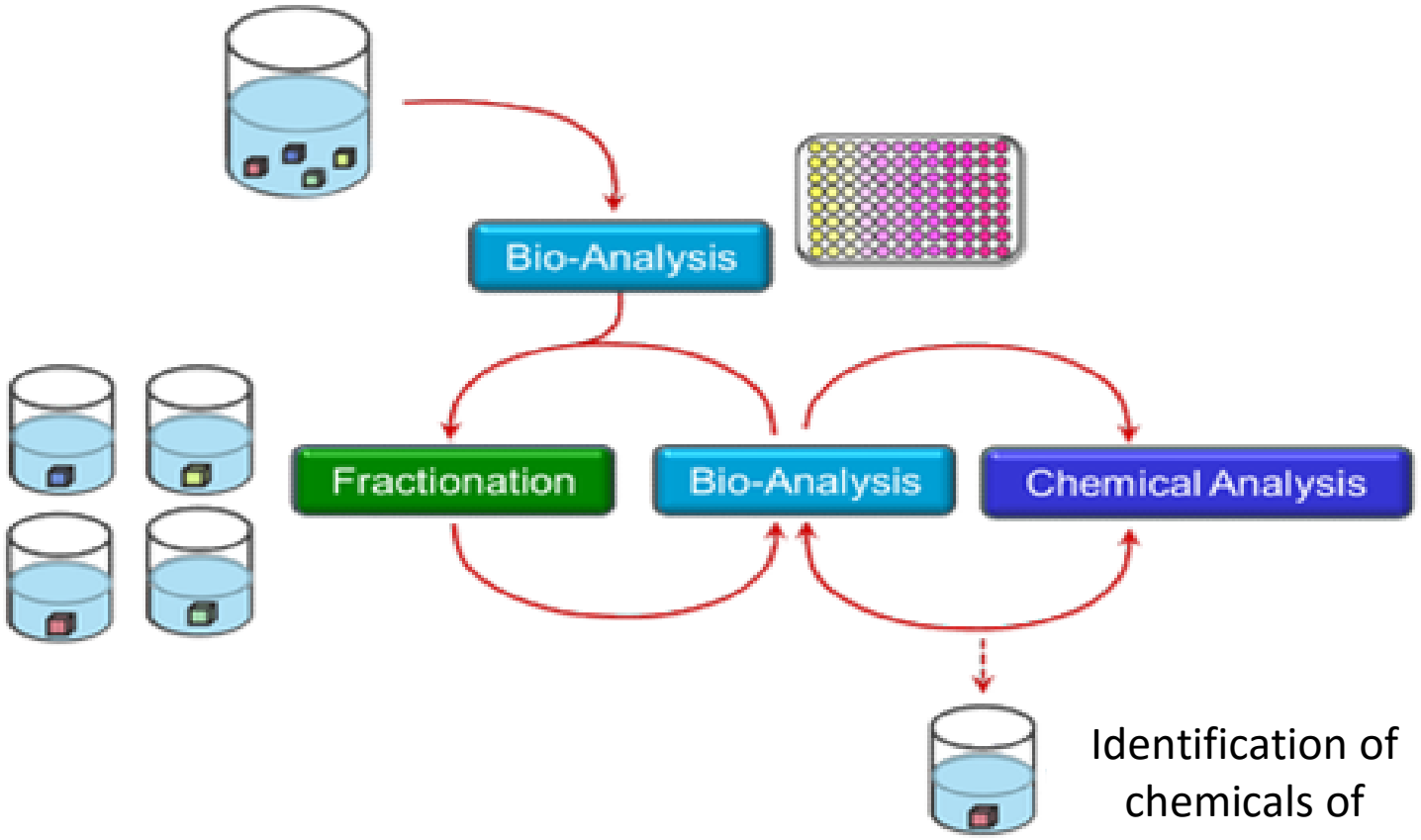


DISCOVERY & TREATMENT OF GLUCOCORTICOID STEROIDS

Jia, A.; Wu, S.; Daniels, K. D.; Snyder, S. A., Balancing the Budget: Accounting for Glucocorticoid Bioactivity and Fate during Water Treatment. *Environ. Sci. Technol.* **2016**.

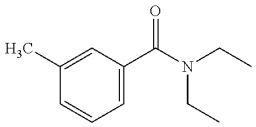
Integrated Approach

Effect Directed Analysis



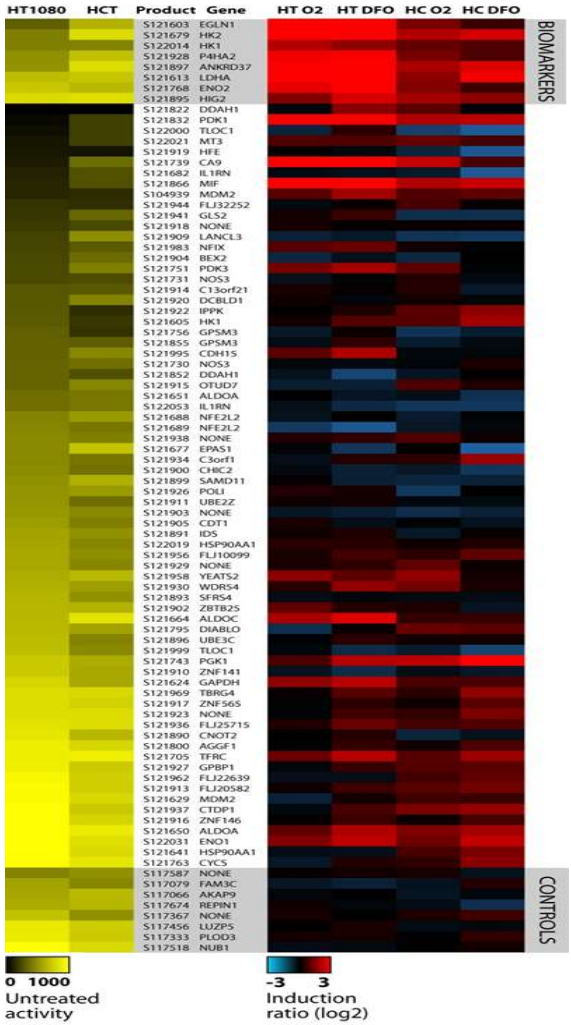
Identification of chemicals of interest

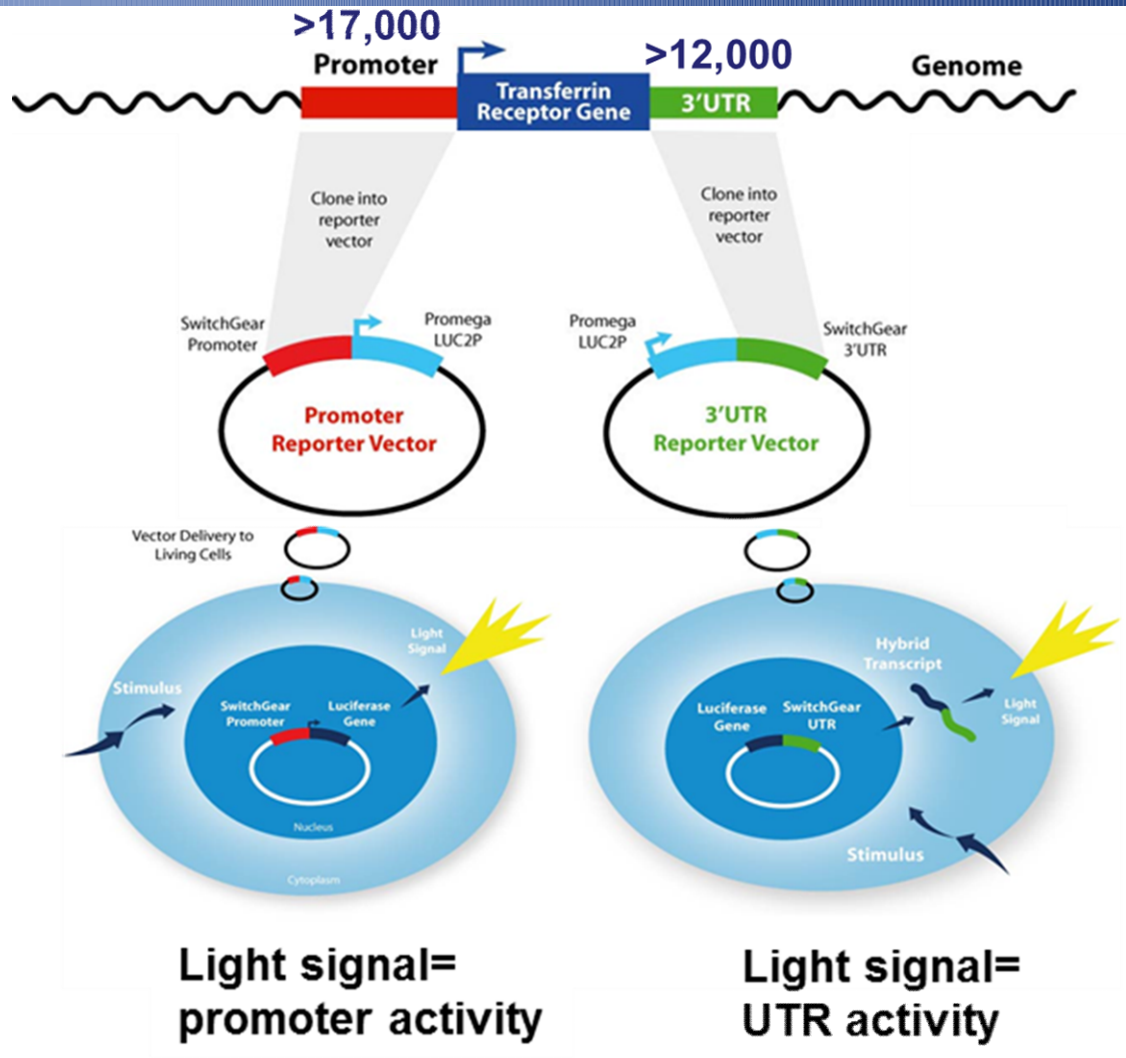
Integrated Approach



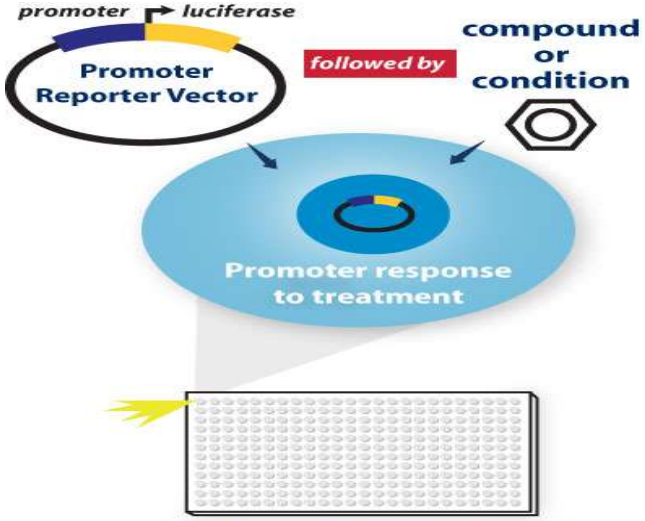
NTA	Bioassays
Develop NTA Tools and Standardizing Protocols	Selection of Endpoints and bioassays
Identify Transformation Products and Other Unknowns	Interpretation Framework for Cell Bioassay Results
Characterize Source Water Quality	Standardization of Methods
Update Surrogate Lists for Targeted Monitoring	Multiplexing Cell Bioassay Technologies

Transient Transfection Assay





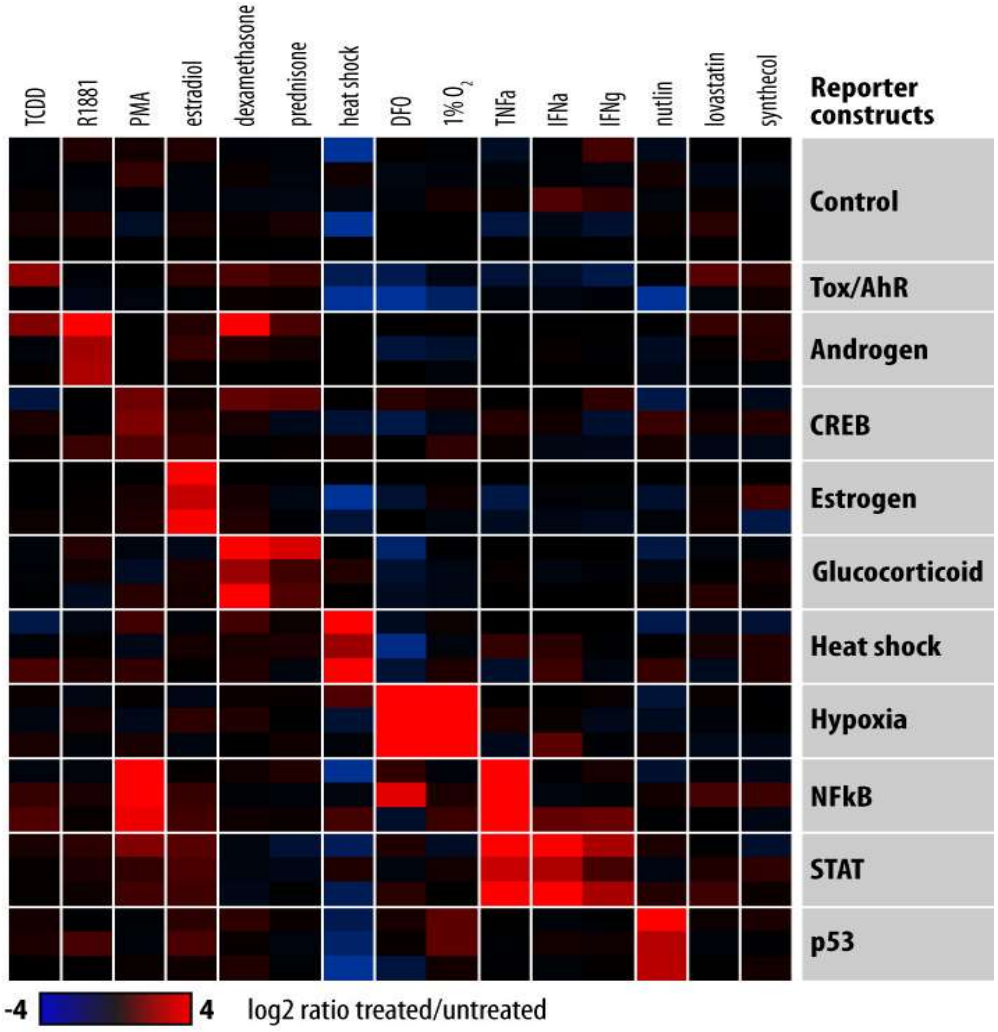
Multiple Endpoints Considered



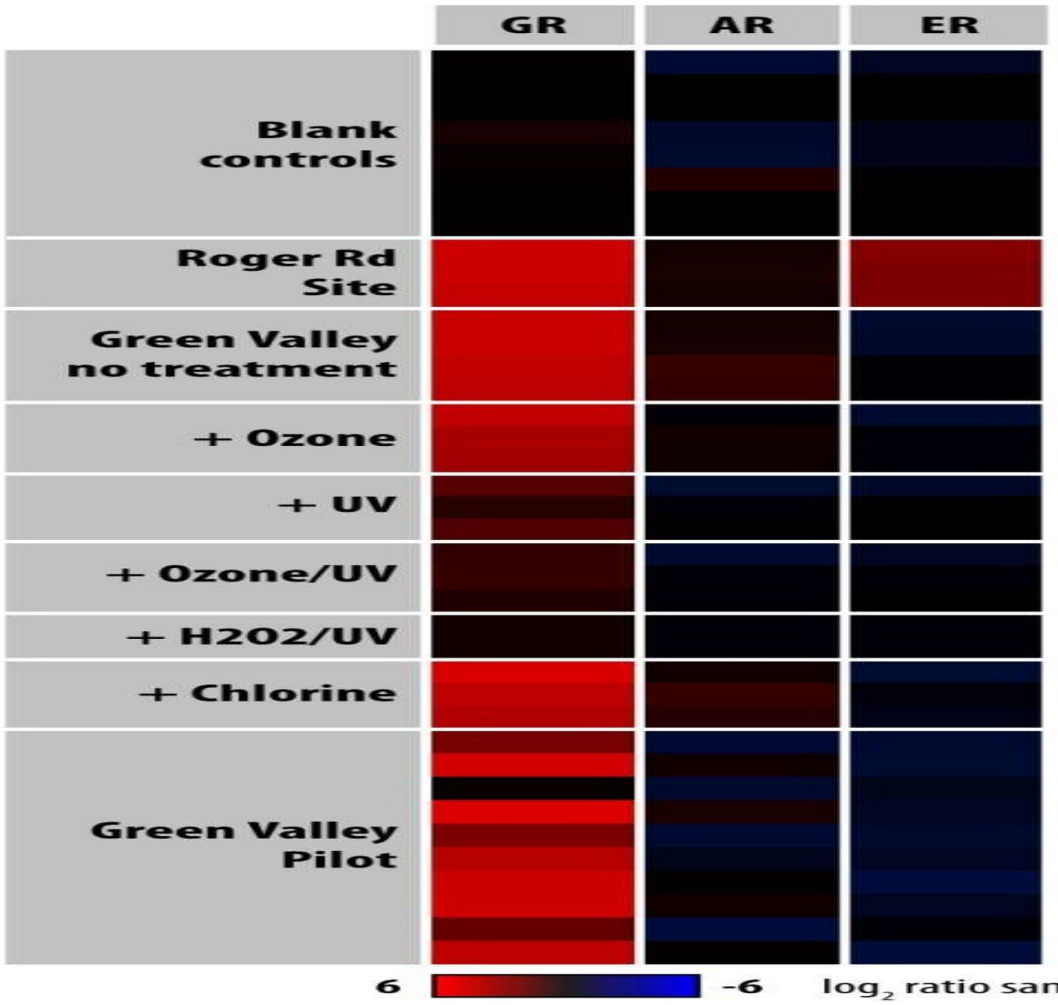
Pathway Profiling Panel
48 promoters and controls

Pathway activity readout for:

HIF1a	Hypoxia
NFkB	Inflammation
CREB	cyclic-AMP
HSF1	Heat shock
p53	DNA damage, apoptosis
STAT	Interferon
SREBP	Cholesterol biosynthesis
ER	Estrogen
AR	Androgen
GR	Glucocorticoid
AhR	Toxicity



Reclaimed Water Screening

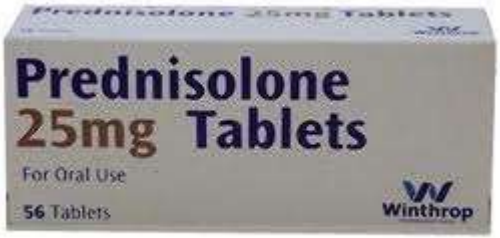


Receptors:

GR = glucocorticoid
AR = androgen
ER = estrogen

Glucocorticoids (GRs)

- Natural & Synthetic
- Used for human diseases such as severe allergies, skin problems, asthma, and arthritis
- Used as veterinary medicine to restore muscle strength and as growth promoters to increase muscle size



GRs Are Widely Used Drugs

Amount prescribed in UK (2006)

Class	Prescribed (kg)
Estrogens	480
Androgens	307
Progestogens	1705
Glucocorticoids	4368

Human and Ecological Risk Assessment: An International Journal

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bher20

Pharmaceuticals in the Aquatic Environment: Steroids and Anti-Steroids as High Priorities for Research

Tamsin J. Runnalls [a] , Luigi Margiotta-Casaluci [a] , Subramaniam Kugathas [a] & John P. Sumpter [a]
[a] Institute for the Environment , Brunel University , Uxbridge, Middlesex, UK
Published online: 15 Dec 2010.

Medicare drugs USA (2013)

TOP 10 MEDICARE TRADITIONAL THERAPY DRUGS

RANKED BY 2013 PMPY SPEND

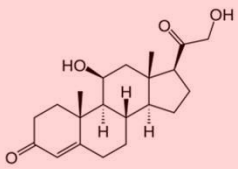
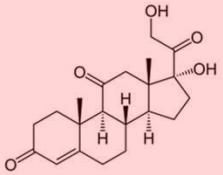
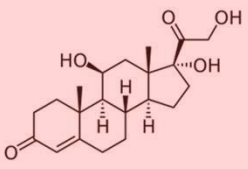
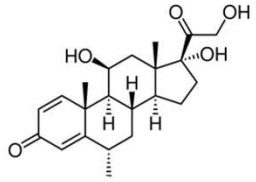
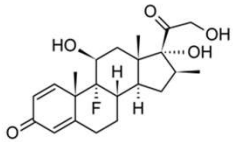
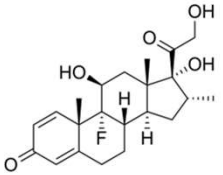
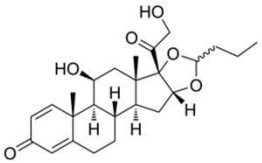
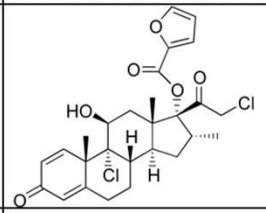
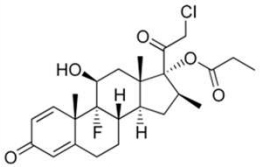
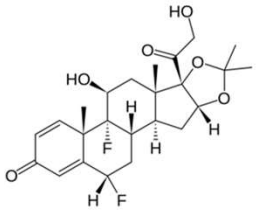
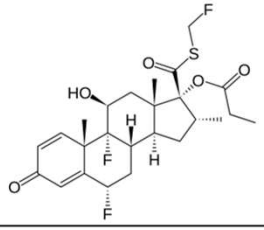
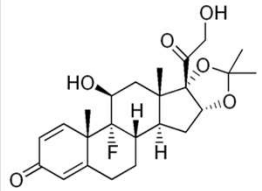
RANK	DRUG NAME	THERAPY CLASS
1	Nexium® (esomeprazole magnesium)	Ulcer Disease
2	Lantus® (insulin glargine)	Diabetes
3	Crestor® (rosuvastatin)	High Blood Cholesterol
4	Advair Diskus® (fluticasone propionate / salmeterol)	Asthma
5	Spiriva® HandiHaler® (tiotropium)	COPD
6	Abilify® (aripiprazole)	Mental / Neurological Disorders
7	Cymbalta® (duloxetine)	Depression
8	Namenda® (memantine)	Mental / Neurological Disorders
9	Januvia® (sitagliptin)	Diabetes
10	atorvastatin	High Blood Cholesterol

Source: The 2013 Drug Trend Report, Express Scripts Lab.

Approved as OTC in 2014

Fluticasone propionate (Flonase)
Triamcinolone acetonide (Nasacort)

GR Chemical Structures

Corticosterone **C21H30O4**	Cortisone **C21H28O5**	Hydrocortisone **C21H28O5**	6α-Methylprednisolone **C22H30O5**
			
Betamethasone **C22H29FO5**	Dexamethasone **C22H29FO5**	Budesonide **C25H34O6**	Mometasone furoate **C27H30Cl2O6**
			
Clobetasol propionate **C25H32ClFO5**	Fluocinolone acetonide **C24H30F2O6**	Fluticasone propionate **C25H31F3O5S**	Triamcinolone acetonide **C24H31FO6**
			

In most synthetic GRs, halogens are incorporated to increase drug stability, potency, and efficacy.

In Vivo Evaluation with GRs

Glucocorticoid activity detected by *in vivo* zebrafish assay and *in vitro* glucocorticoid receptor bioassay at environmental relevant concentrations

Chemosphere 144 (2016) 1162–1169

Qiyu Chen [a], Ai Jia [b], Shane A. Snyder [b], Zhiyuan Gong [c], Siew Hong Lam [a, c, *]

Treatment in Larvae / Gene	Dexamethasone (DEX)				Prednisolone (PRE)				Triamcinolone (TRI)			
	50 pM	500 pM	5 nM	50 nM	50 pM	500 pM	5 nM	50 nM	50 pM	500 pM	5 nM	50 nM
pepck	**	**	**	**	*	**	**	**	**	**	**	**
baiap2	**	**	**			**	**	**	**	**	**	**
pxr	**			**			**	**	**	**	**	**
mmp-13	**					**	**	*		**	**	**
cdkn1c				**	*				**	**		**
mmp-2							*			*	*	**
fkbp5				*								
cyp3a65						*						
sox9b		**		**			**					
gilz		*					*					
mmp-9						**	**	**				

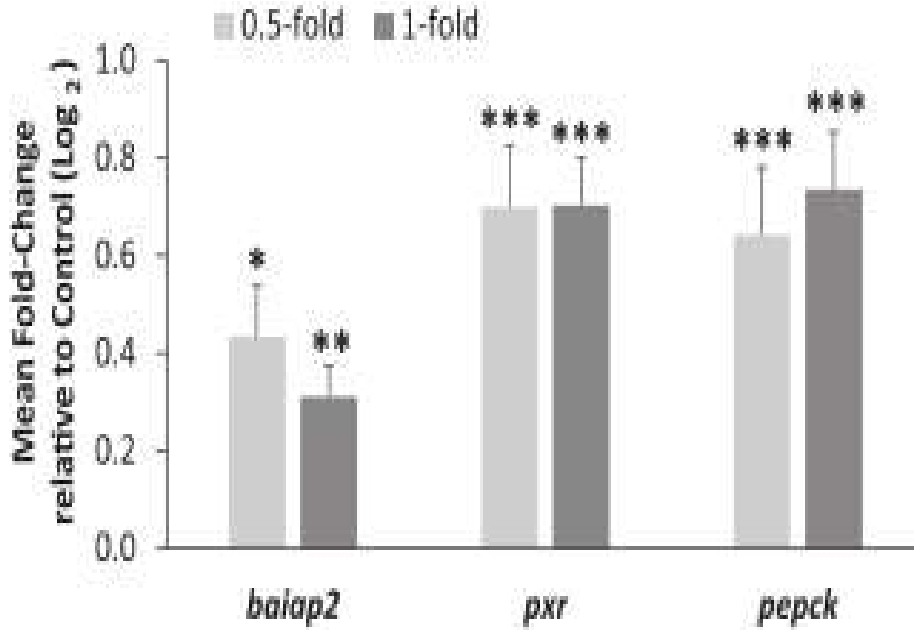
Mean Fold-Change relative to Control (Log_2)

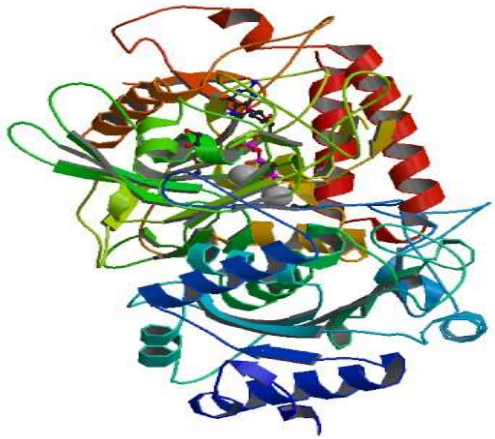
- > 3
- > 2 to 3
- > 1 to 2
- > 0 to 1
- Not significant (P > 0.05)
- < 0 to -1
- < -1 to -2
- < -2

** P < 0.01

* P < 0.05

In Vivo Evaluation with GRs





pepck enzyme

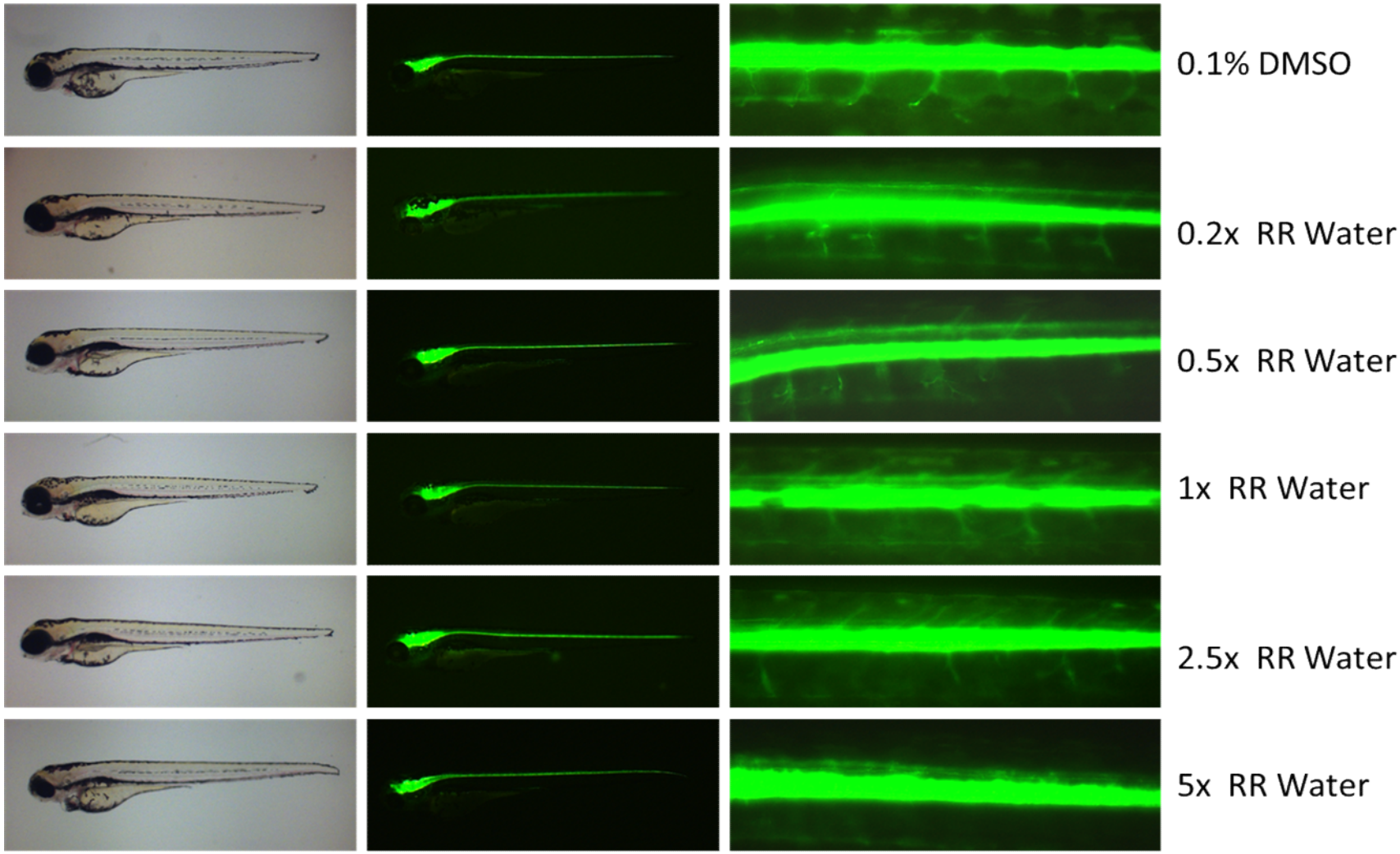
Brain-specific angiogenesis inhibitor 1-associated protein 2): insulin receptor

Pregnane X receptor (pxr): signals for detoxification

Phosphoenolpyruvate carboxykinase (**pepck**): gluconeogenesis

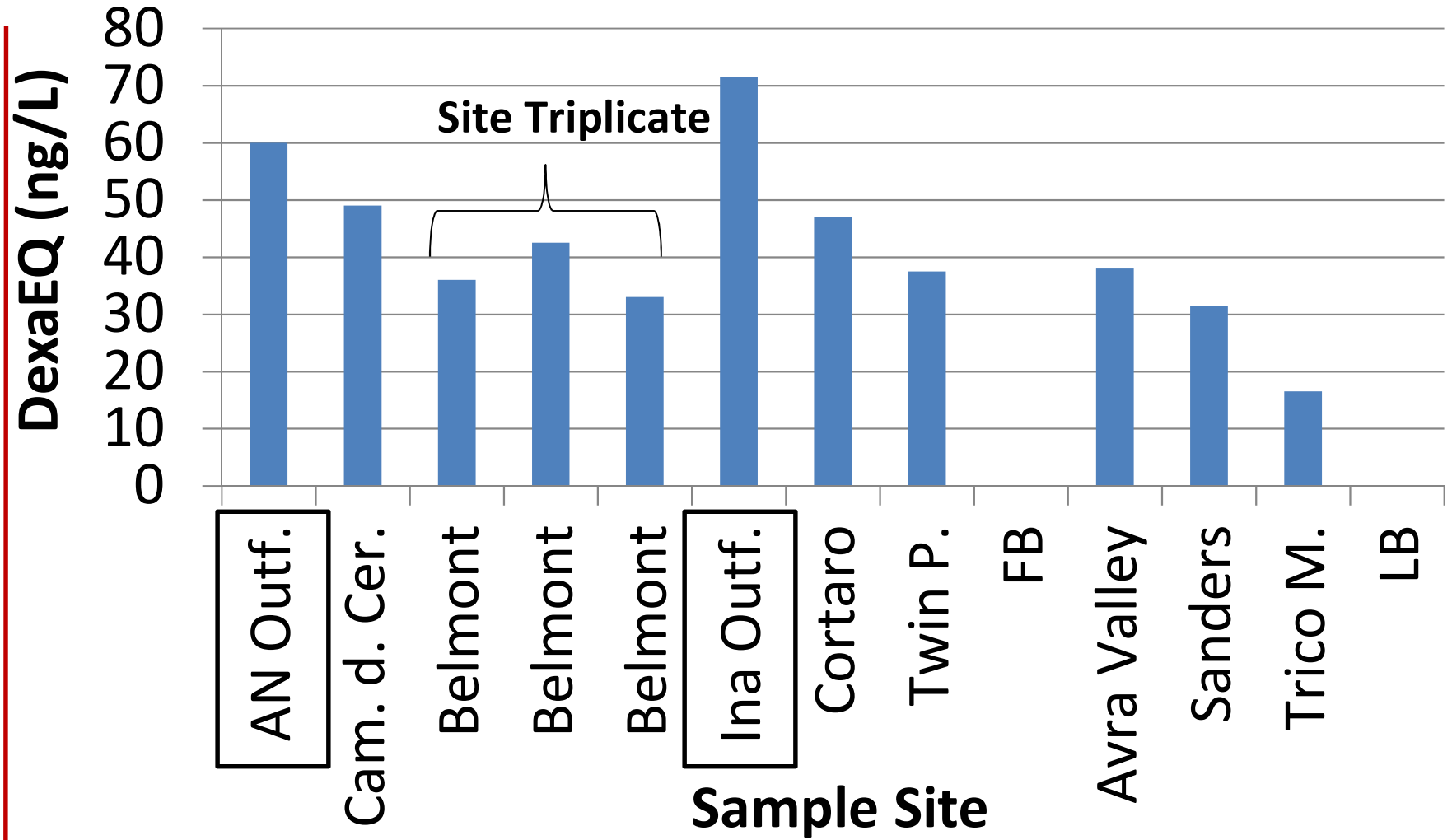
Chemosphere 144 (2016) 1162–1169

In Vivo Evaluation with GRs

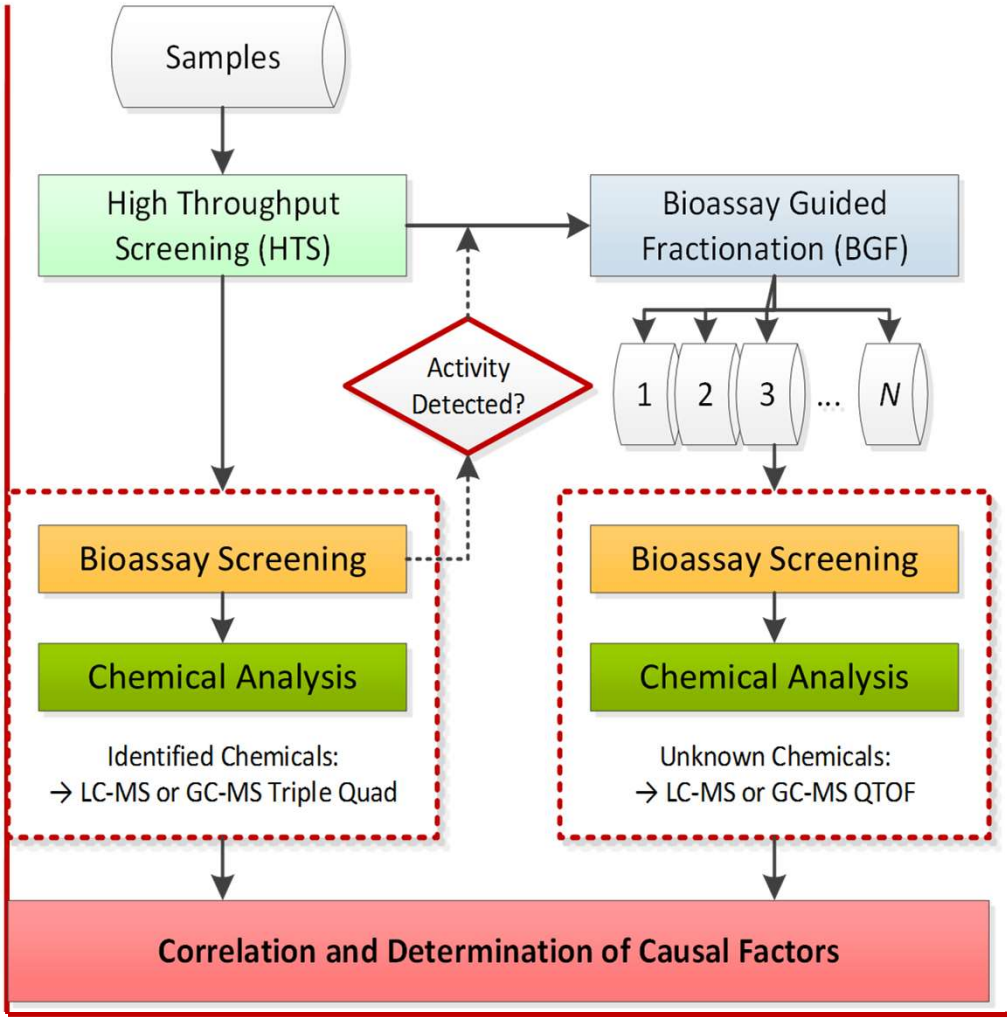


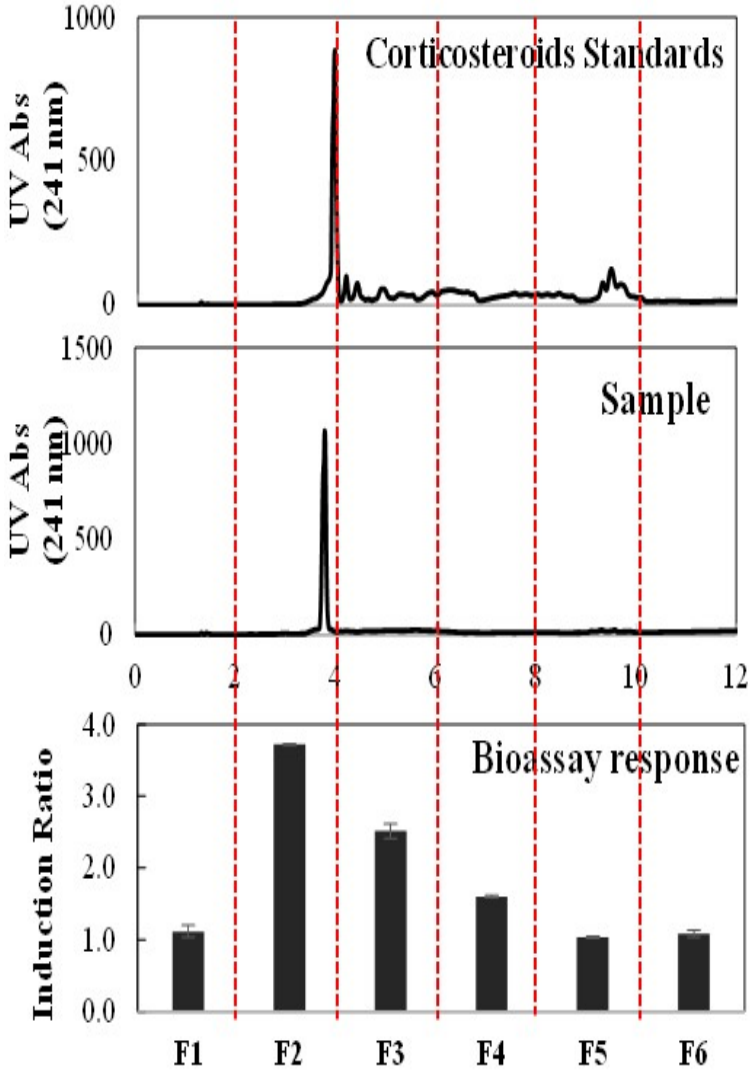
Santa Cruz River Sampling Sites

GR Cellular Activity



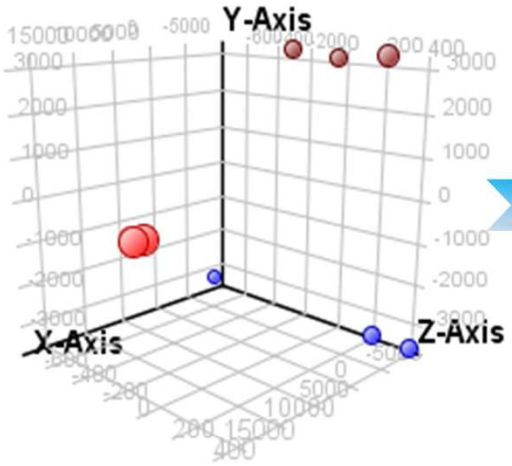
Fractionation and Bioassay





QTOF Analysis

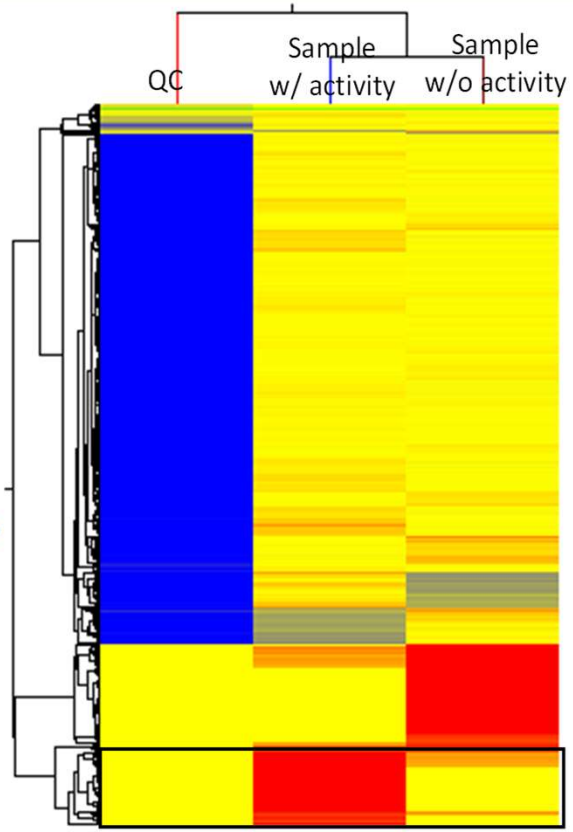
Principal Component Analysis



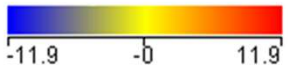
QC

Sample w/ activity

Sample w/o activity

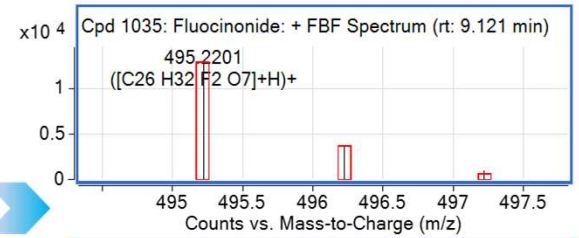


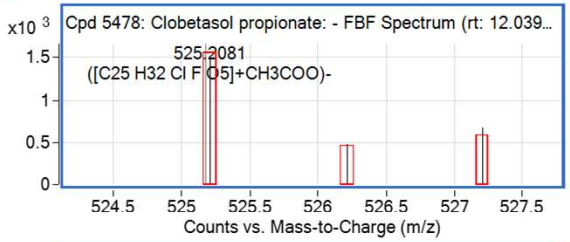
Color by normalized abundance

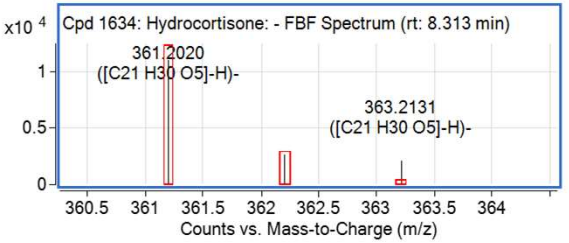


Datadase Search &

Compounds Identification

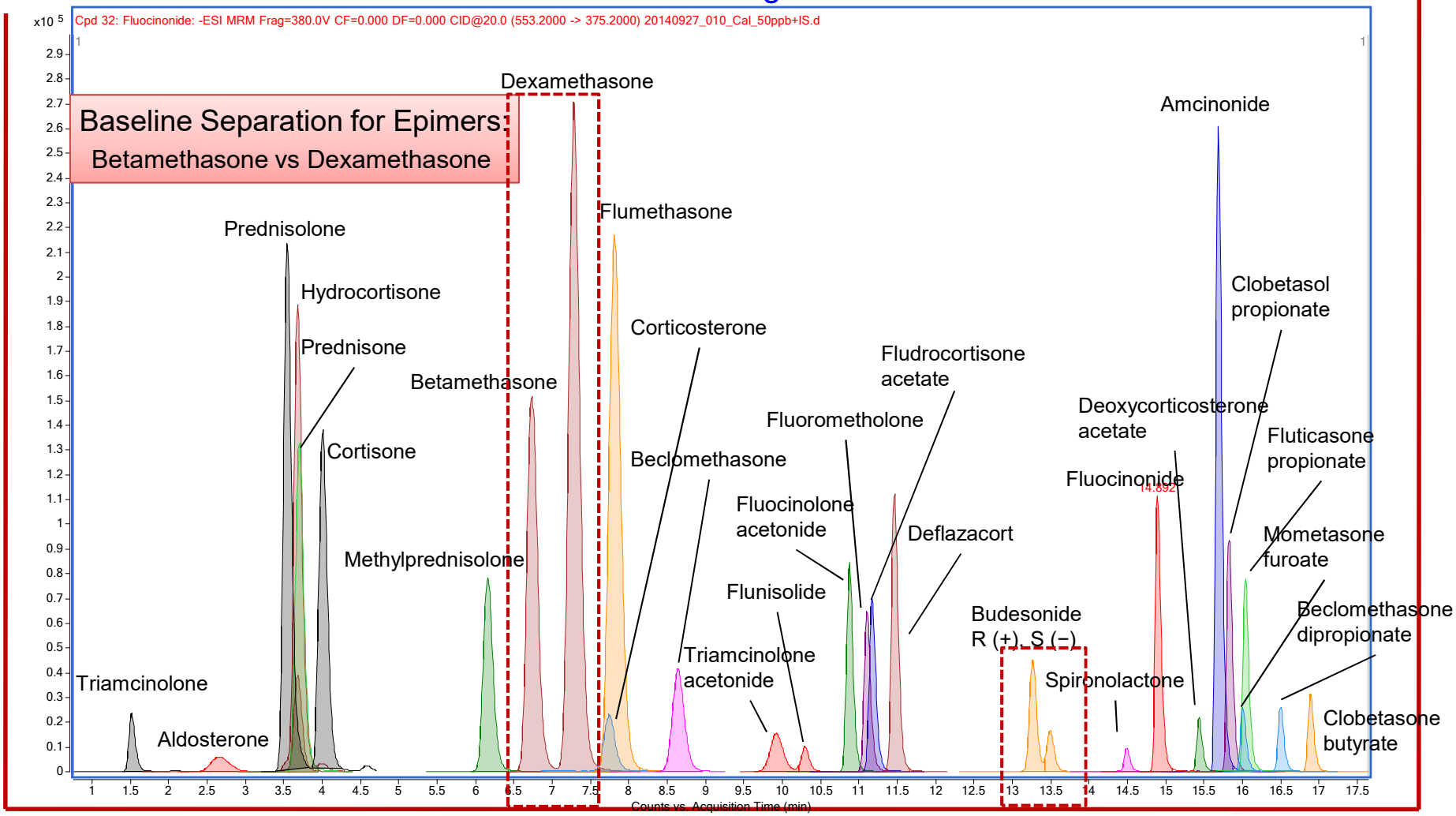






Targeted Quantitative Analysis

MDLs: 0.02-5 ng/L



Balancing the GR Budget

WWTP Effluents

In vitro GR bioassay

Occurrence

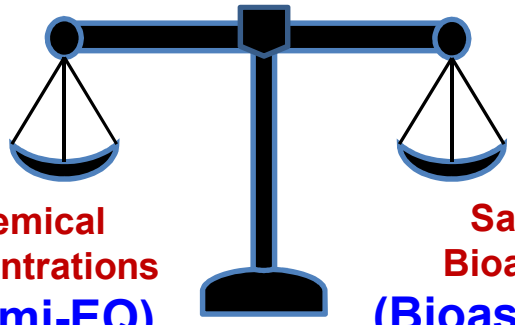
Reclamation Treatment

LC-MS/MS Analysis

27 agonists

Attenuation

- Chlorination
- Ozone
- UV
- Membrane



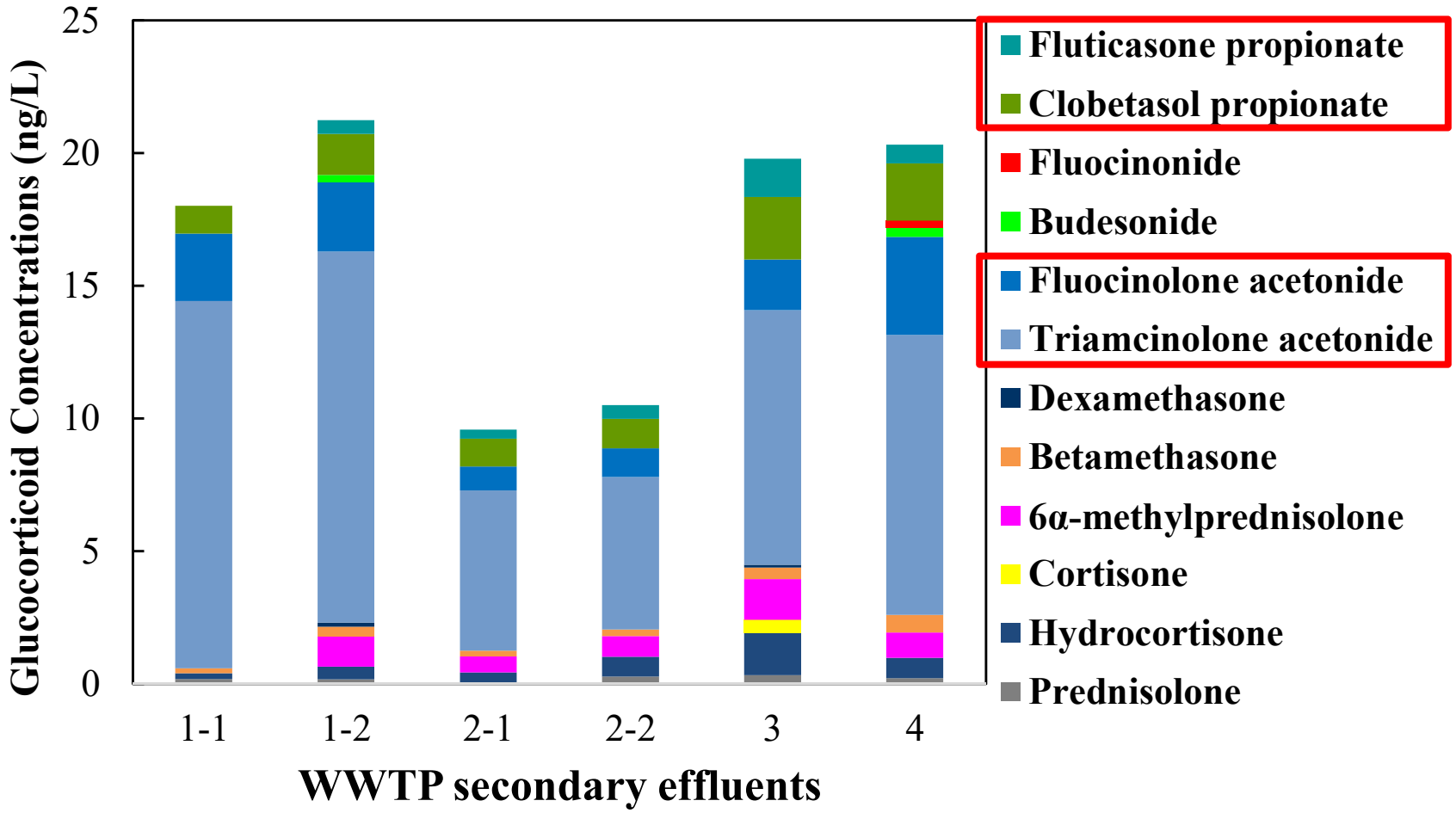
Chemical Concentrations (Chemi-EQ)

Sample Bioactivity (Bioassay-EQ)

Treatment Fate

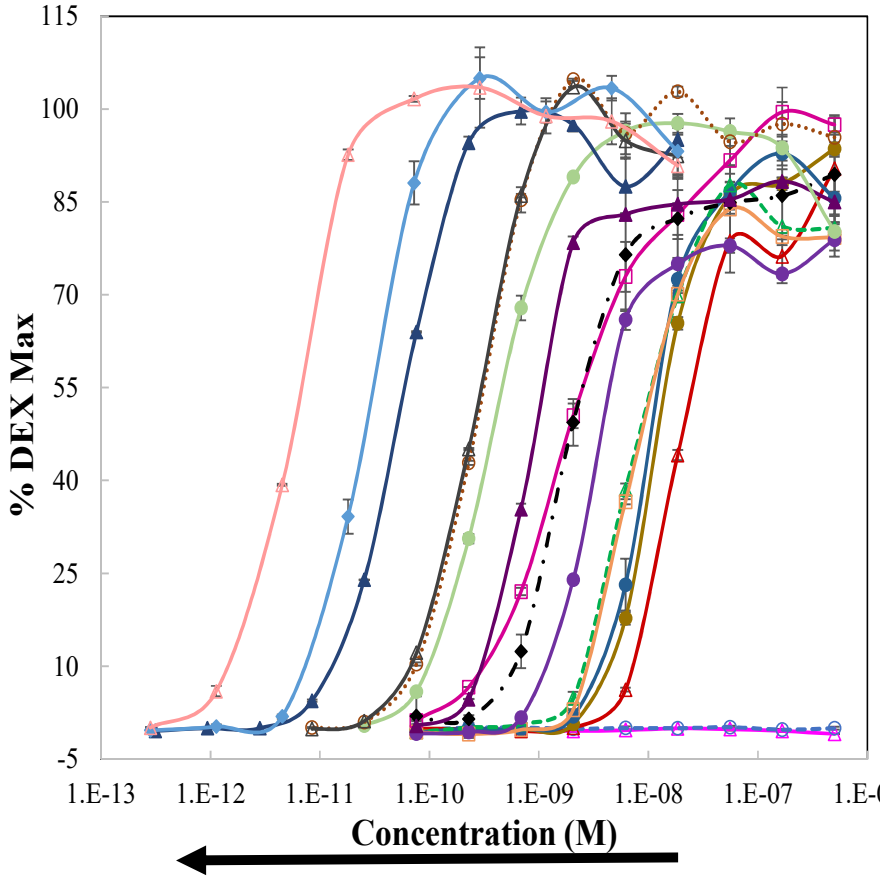
Toxicity Reduction

GR Occurrence



9.6-21.2 ng/L, much higher occurrence levels than estrogens at same sites

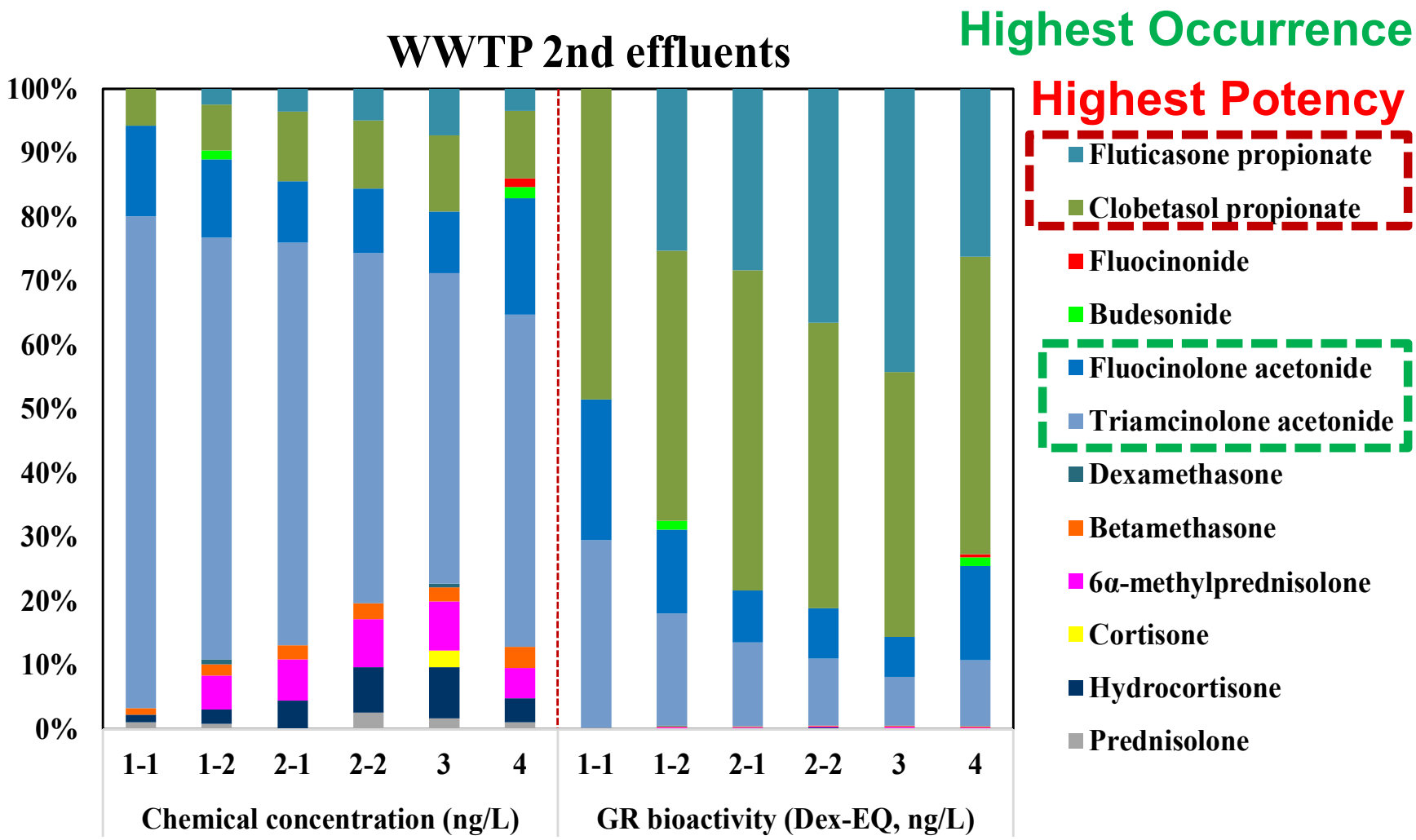
Biological Potency (In Vitro)



GRs	EC_{50} (nM)	REP
Prednisone	>500	<0.004
Cortisone	>500	<0.004
Prednisolone	17.7	0.101
Triamcinolone	11.8	0.152
Fludrocortisone acetate	9.67	0.185
Hydrocortisone	6.81	0.264
6α-methylprednisolone	6.79	0.264
Betamethasone	2.83	0.634
Fluocinonide	1.89	0.948
Dexamethasone	1.79	1.000
Triamcinolone acetonide	0.79	2.265
Flumethasone	0.36	5.032
Budesonide	0.26	6.895
Fluocinolone acetonide	0.24	7.398
Clobetasol propionate	0.048	37.04
Fluticasone propionate	0.025	70.88
GRs mixed standard	0.005	329

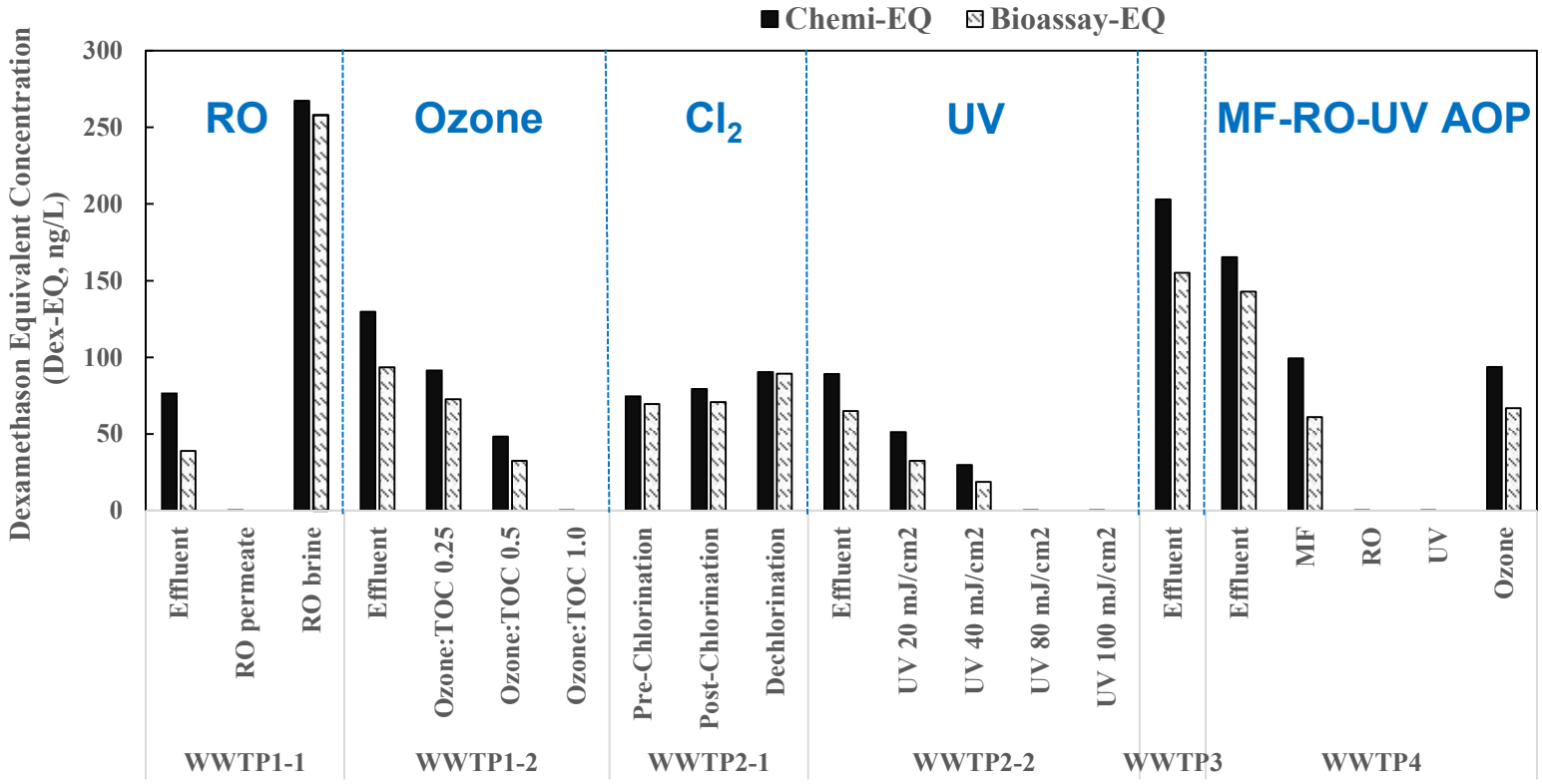
Synthetic GRs have much higher bioactivity.

GR Concentration and Activity

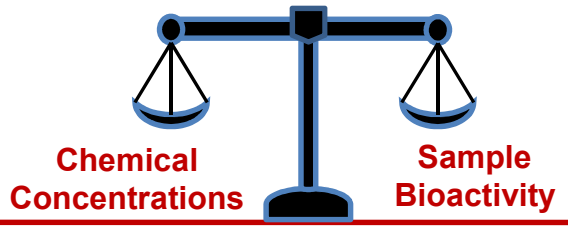


Mass-Activity Balance Achieved

Chemi-EQ=Sum(concentration*relative potency)



The quantitated GRs can explain the observed biological GR activity.



Acknowledgements

ARIZONA

SNYDER RESEARCH GROUP

PIONEERING RESEARCH REGARDING DETECTION, TREATMENT, AND HEALTH RELEVANCE OF ENVIRONMENTAL CONTAMINANTS

Agilent Technologies

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