



GC-qTOF Analysis of Glucocorticoid Transformation Products in Water

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NEMC Conference, July 13, 2015



Outline

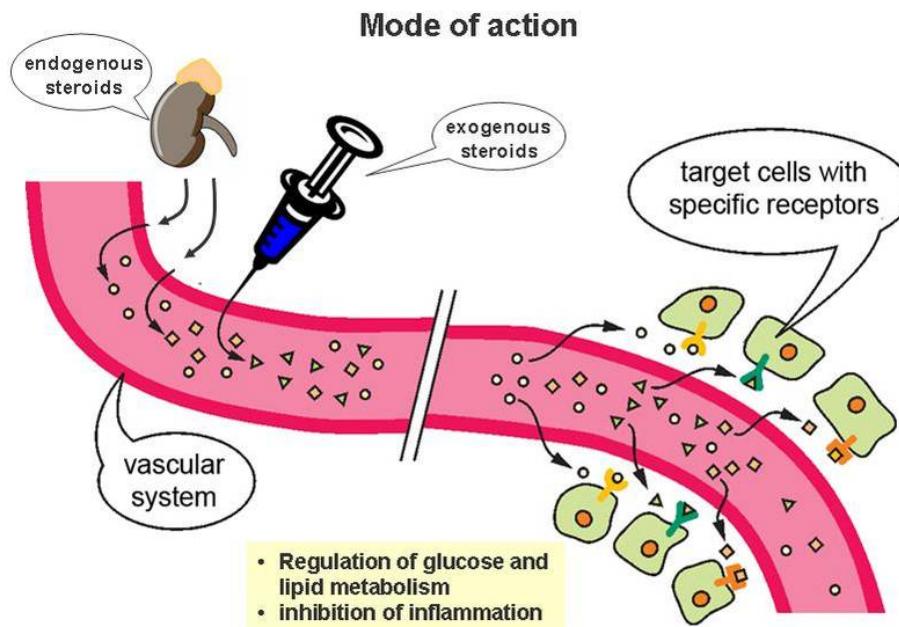
- ❖ Introduction
- ❖ Aim
- ❖ Methodology
- ❖ Results
- ❖ Summary
- ❖ Future Direction
- ❖ Questions





What are glucocorticoids (GRs)?

- Class of steroid hormones
- Naturally-occurring and synthetically produced
- Cortisol is major natural GR
- Regulates development, aging and adaptation to stress





Synthetic GRs

Used to treat asthma, allergies, arthritis, muscle strength, cancer, and autoimmune diseases

- Cushing Syndrome
- Addison's Disease

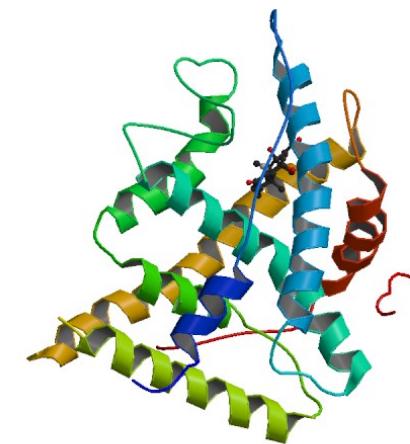
It is estimated that 1.2% of the U.S. population use glucocorticoid medications long-term (2012)





Health Implications

- **Metabolic effects¹ –**
Carbohydrate & protein metabolism
- **Protein expression¹ –**
Binds to the GR receptor, regulating expression of anti-inflammatory protein
- **Physiological effects² –**
Immunosuppression, hyperglycemia, osteoporosis, adrenal suppression



¹Overman et al., 2013 ²Kugathas et al., 2013



Presence in Wastewater

Intake by humans and animals



Excretion through urine

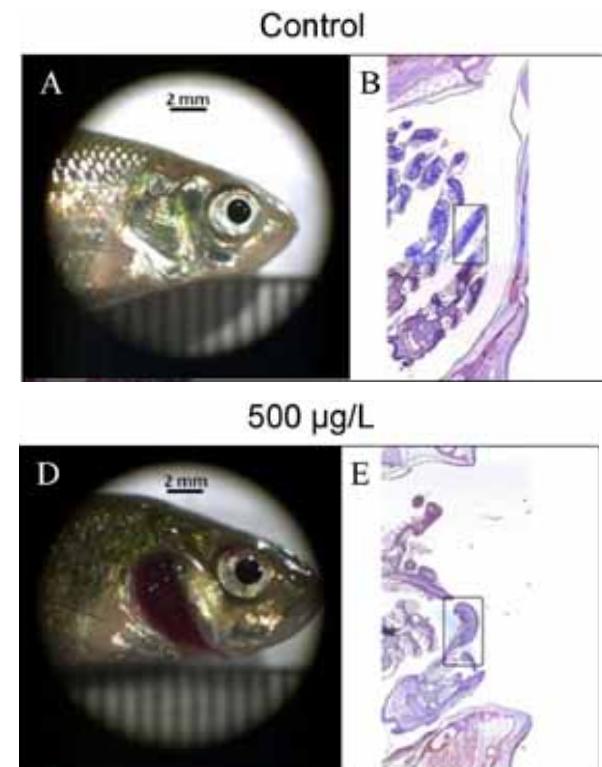


Released into environment through
wastewater treatment process



Implications- Aquatic Life

- **Ecotoxicological effects¹ –**
Inhibited locomotion,
aggressive behavior, and
deformities
- **Reproductive effects³-**
Induced masculinization and
impaired reproduction

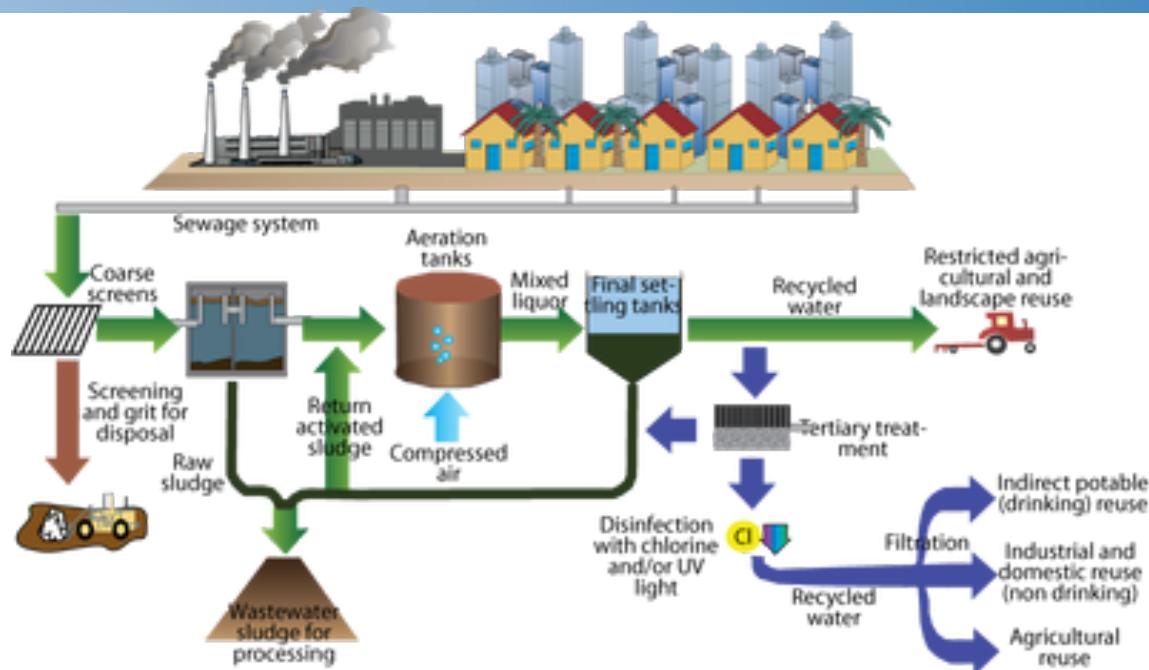


Figure². Gill histopathology of control (top) and Dexamethasone-treated (bottom) fish.

¹Chang et al., 2007 ²Kugathas et al., 2013 ³LaLone et al., 2011



Removal of GRs from WWTP



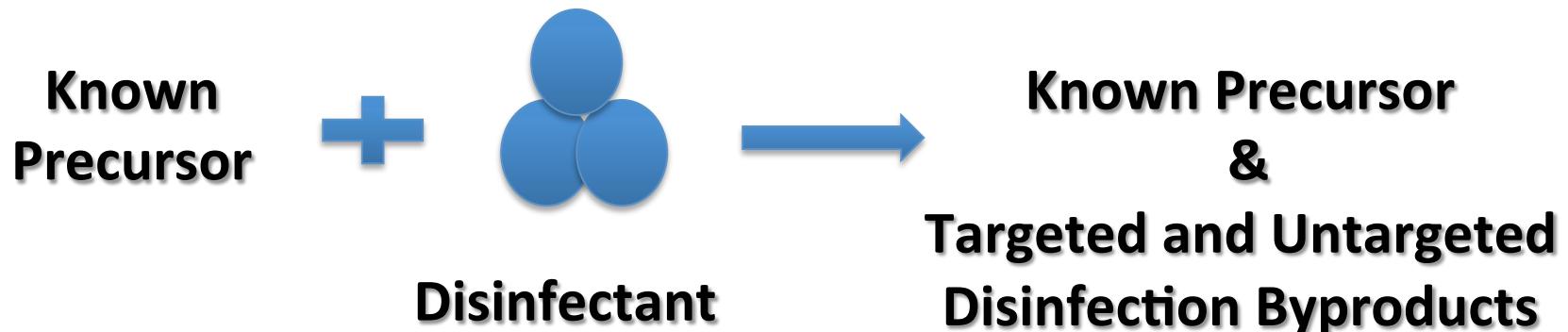
Figure¹. Illustration of WWTP process

- 171 ng/L average GR conc. in influent from 6 WWTPs in China²
- 90% removal in WWTP using sludge/ chlorine treatment and 80% removal in plant with oxidation/ UV treatment³
- <10 ng/L of glucocorticoid steroids in effluent³
- 1.55 ng DEX eq/L avg. glucocorticoid activity reported in surface water in Netherlands⁴

¹ian.umcs.edu ²Chang et al., 2007 ³Liu et al., 2012 ⁴Schricks et al., 2013



Disinfection Byproducts (DBPs)



- Free Chlorine and Monochloramine are the most common disinfectants
- THMs are DBPs of greatest abundance and only a few are regulated by the U.S. EPA
- Monochloramine produces fewer THMs, but produces nitrosamines
- The presence of bromide and iodide leads to more toxic DBPs



Aim

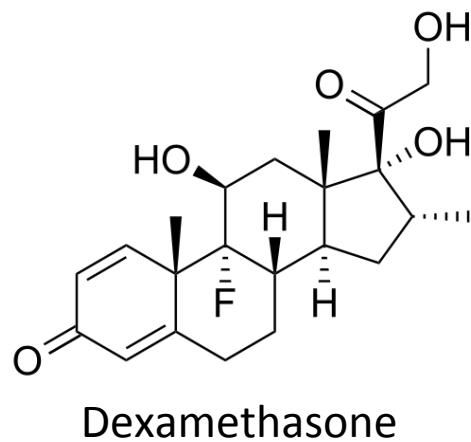
Problem: There are currently no published studies on the transformation of glucocorticoids during disinfection and the resulting biological effects.

Objective: To use the high resolution GC-QToF to identify the transformation products of a glucocorticoid steroid after disinfection and evaluate the effects on glucocorticoid receptor activity.



Dexamethasone

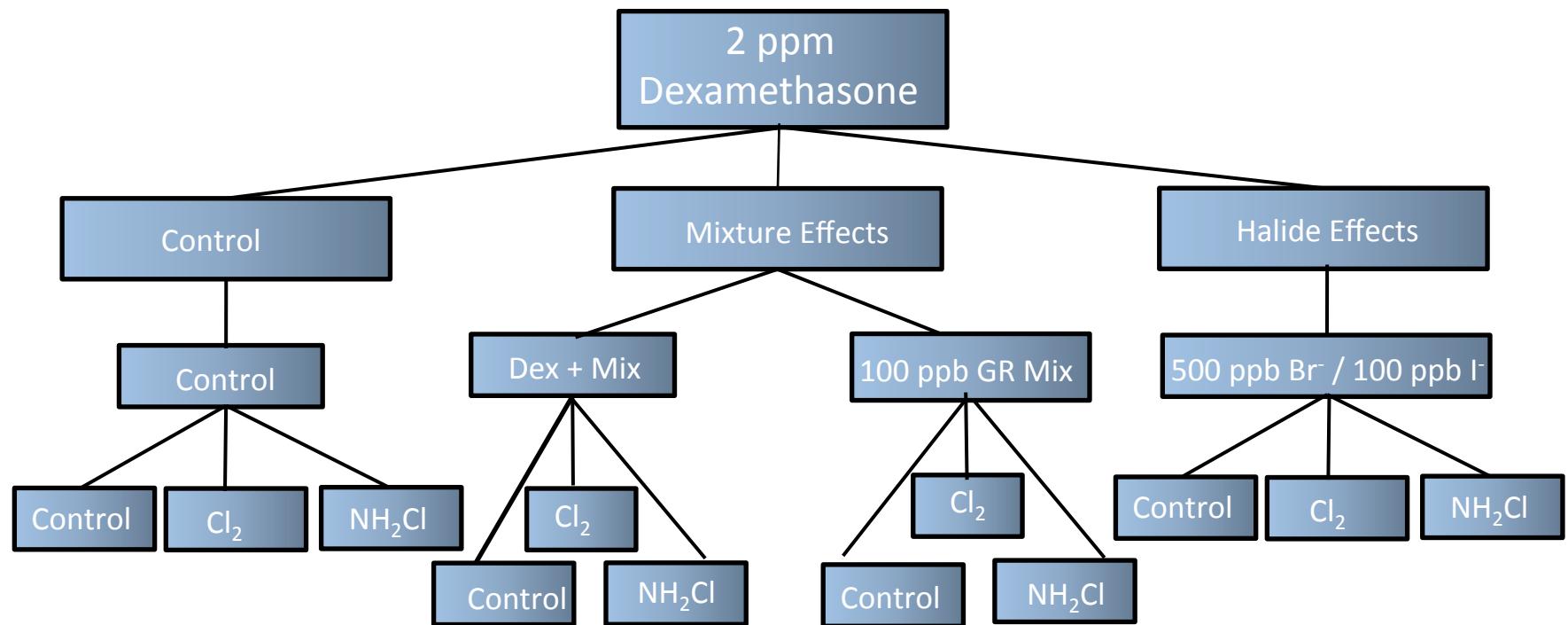
- ❖ 25% more potent than cortisol in glucocorticoid effect¹
- ❖ Glucocorticoid Receptor Agonist²
- ❖ A study has shown that it reduced fecundity and increased female plasma estradiol in fathead minnow²
- ❖ Acceptable Daily Intake (ADI) of 1 $\mu\text{g kg}^{-1}$ bw d⁻¹³
- ❖ Detected in wastewater effluent at > 0.2 ng L⁻¹⁴



¹ Goulding et al., 2001 ² Lalone et al., 2012 ³ JECFA, 1998 ⁴ Chang et al., 2007



Sample Treatment Matrix



pH 6.9 phosphate buffered DI Water

Disinfectant Dose: 4 ppm

Quenched with 40 ppm Sodium Bisulfite after 24 hours



GR Mixture

100 ppb Addition

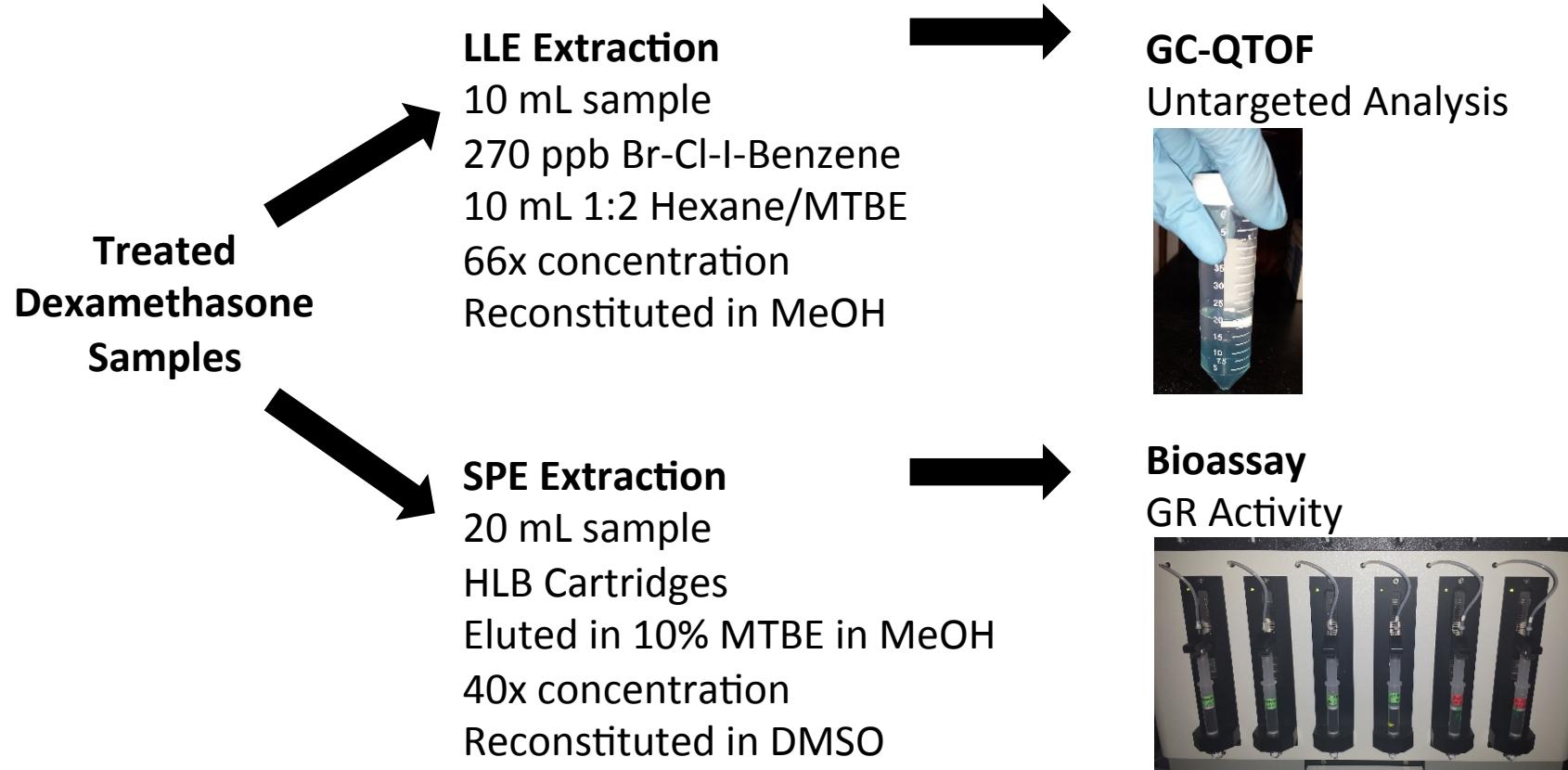
Corticosterone
Cortisone
Deflazacort
Deoxycorticosterone acetate
Dexamethasone
Fludrocortisone acetate
Flumethasone
Flunisolide

Fluocinolone acetonide
Fluocinonide
Fluorometholone
Fluticasone propionate
Hydrocortisone
Methylprednisolone
Mometasone furoate

Prednisone
Spironolactone
Triamcinolone
Triamcinolone acetonide



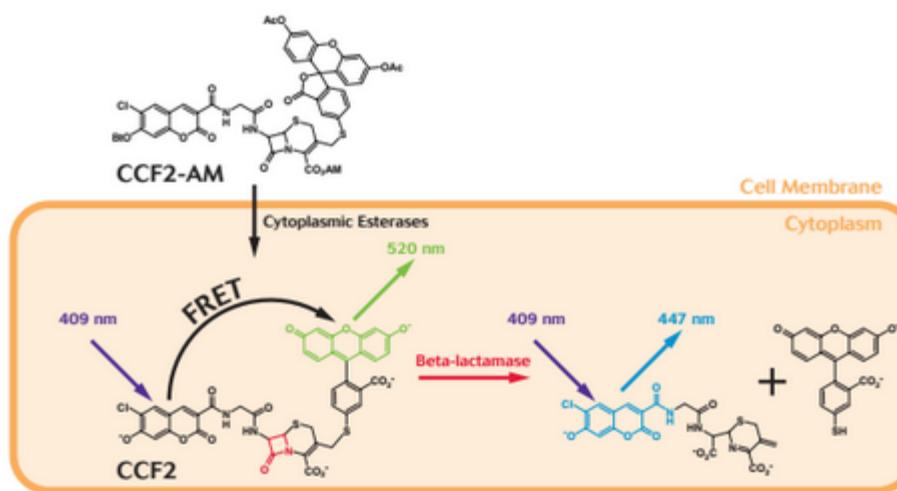
Work Flow





GR UAS-bla-HEK293T Cell-based Bioassay

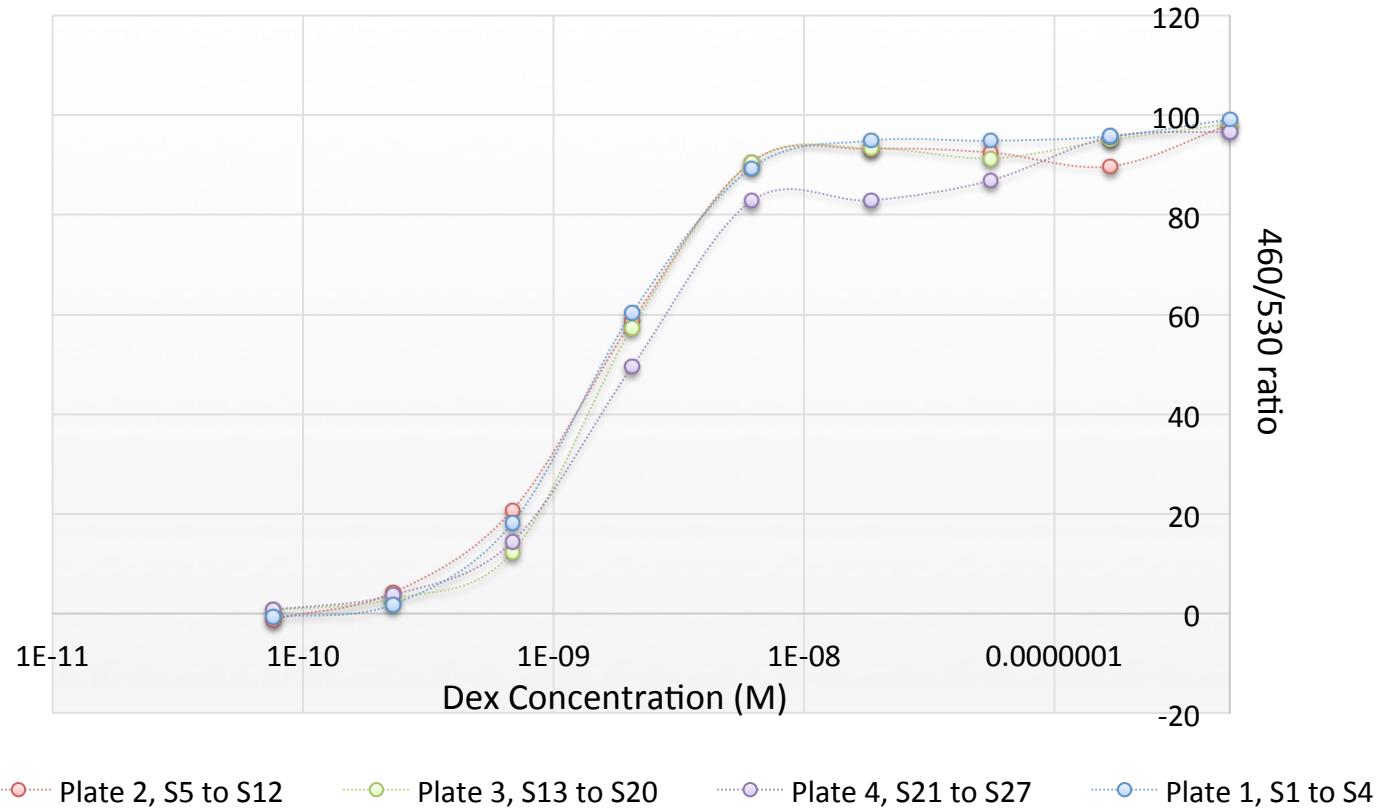
Contains a glucocorticoid receptor ligand-binding domain/ GA1
Beta-lactamase reporter gene/ GA14 DNA binding
Measure of glucocorticoid activity





Dose Response Curve

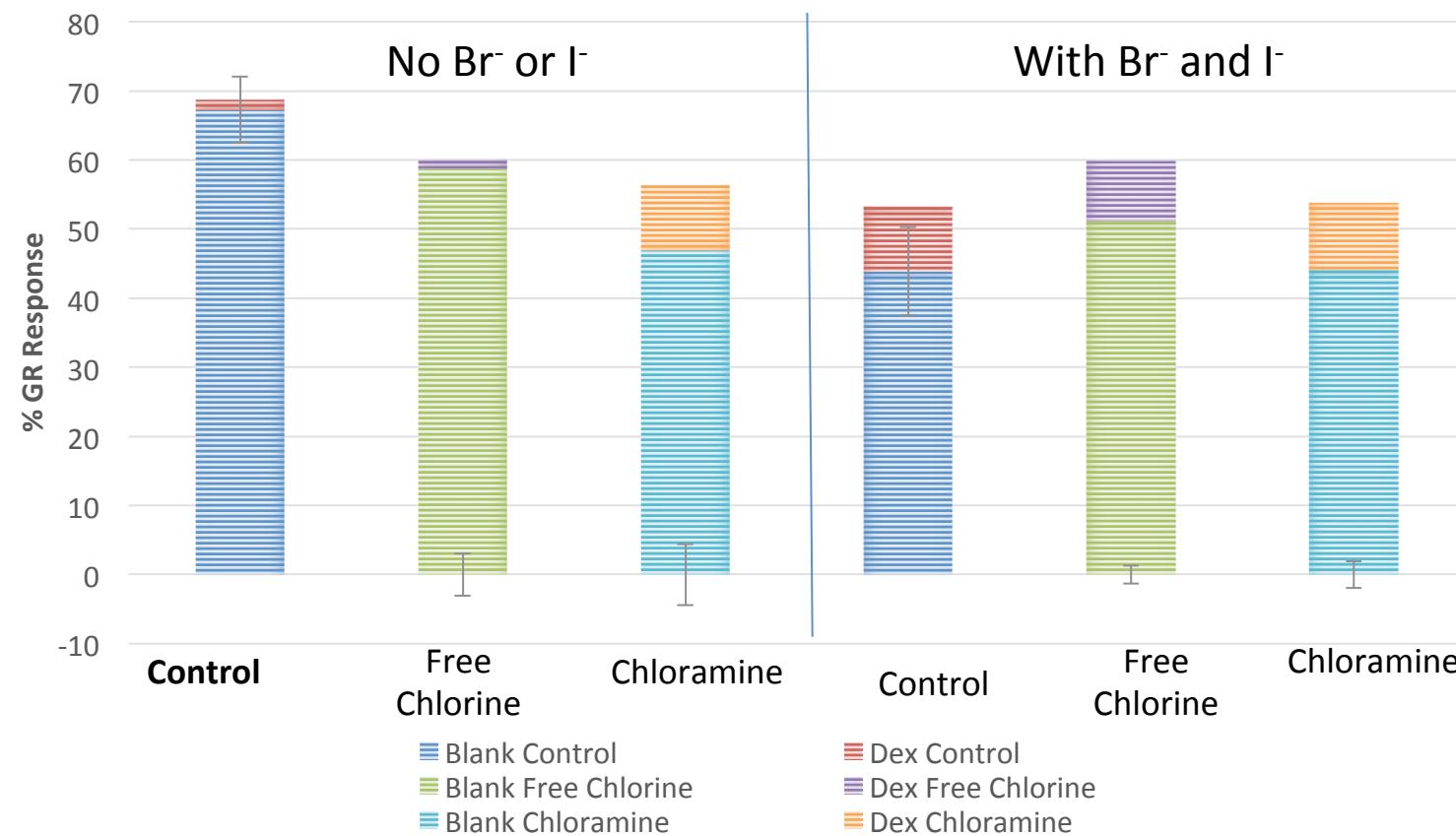
Dose response of
GR-UAS-bla HEK 293T to dexamethasone





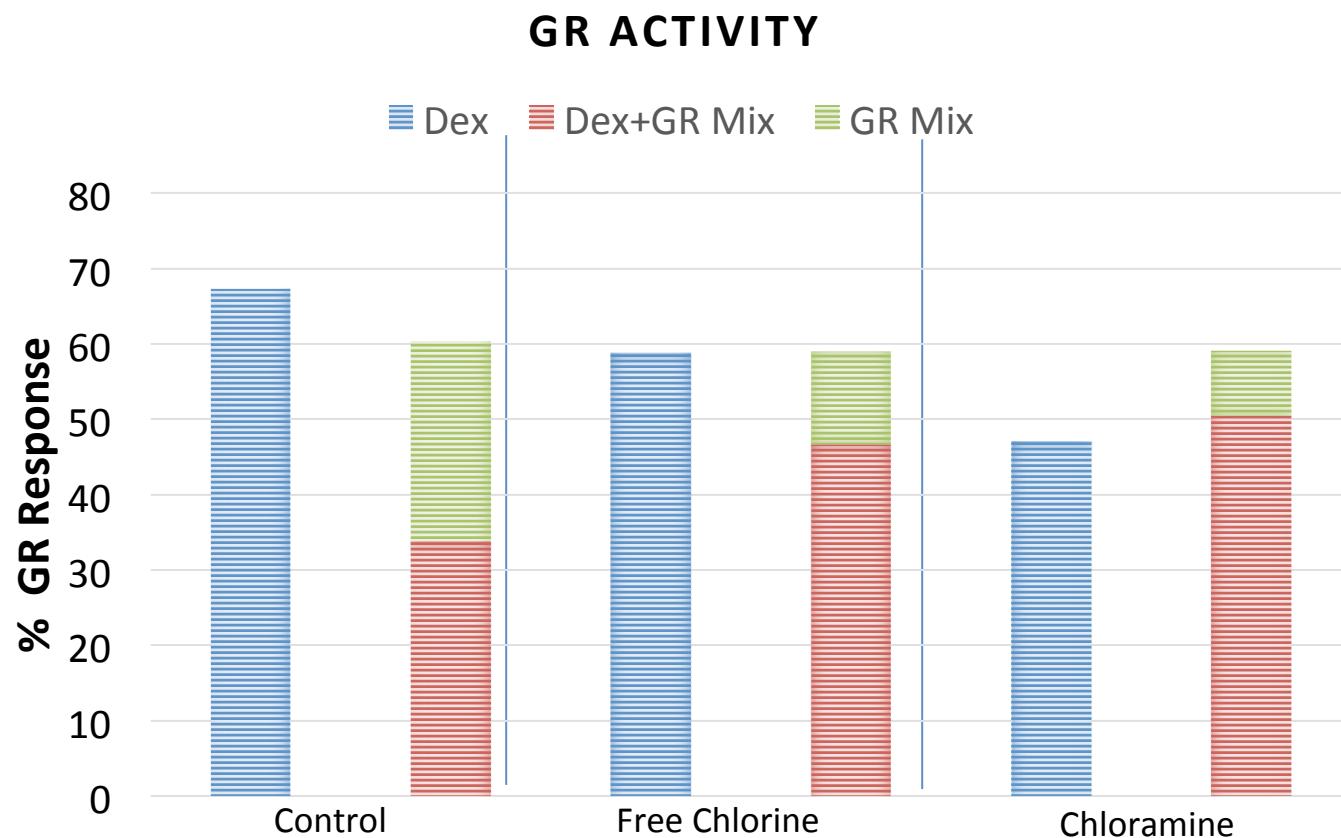
Br⁻ / I⁻ Effects

GR ACTIVITY





Mixture Effects





GC-qTOF/MS Untargeted Analysis

Agilent 7890A GC/7200 QToF MS

GC Conditions

Column- DB-5 MS UI (30m x 250 μ m x 0.25 μ m)

Injector Mode- Splitless

Injection Volume- 1 μ L

MS Conditions

Ionization Mode- EI (70eV)

Type of Data Acquisition- MS Only

Source Temp- 230°C

Quad Temp- 150°C

Emission Current- 35A°

Acquisition Mode- 2 GHz

Acquisition Range- 40-800 amu

Acquisition Speed- 5 spectra/ second



Figure. Shown above is the Agilent 7200 GC/Q-TOF. It is equipped with the PAL autosampler.



GC-ICP/MS Confirmation

Agilent 7890B GC/7900 ICP MS

GC Conditions

Same as GC-qTOF

ICP-MS Conditions

Dwell times (seconds)

C-12 0.1500

C-13 0.4000

C-35 0.8000

Br-79 0.5000

Br-81 0.5000

I-127 0.1500

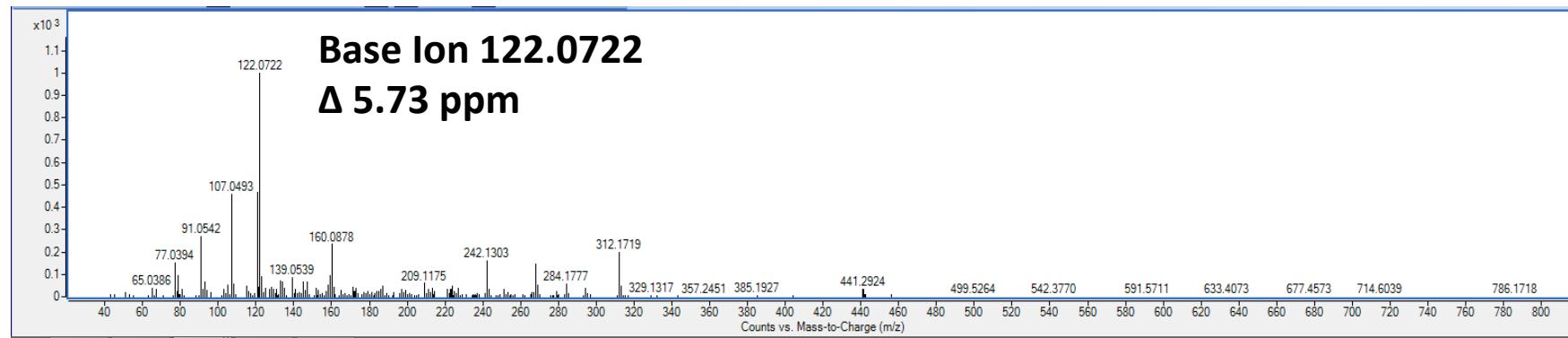
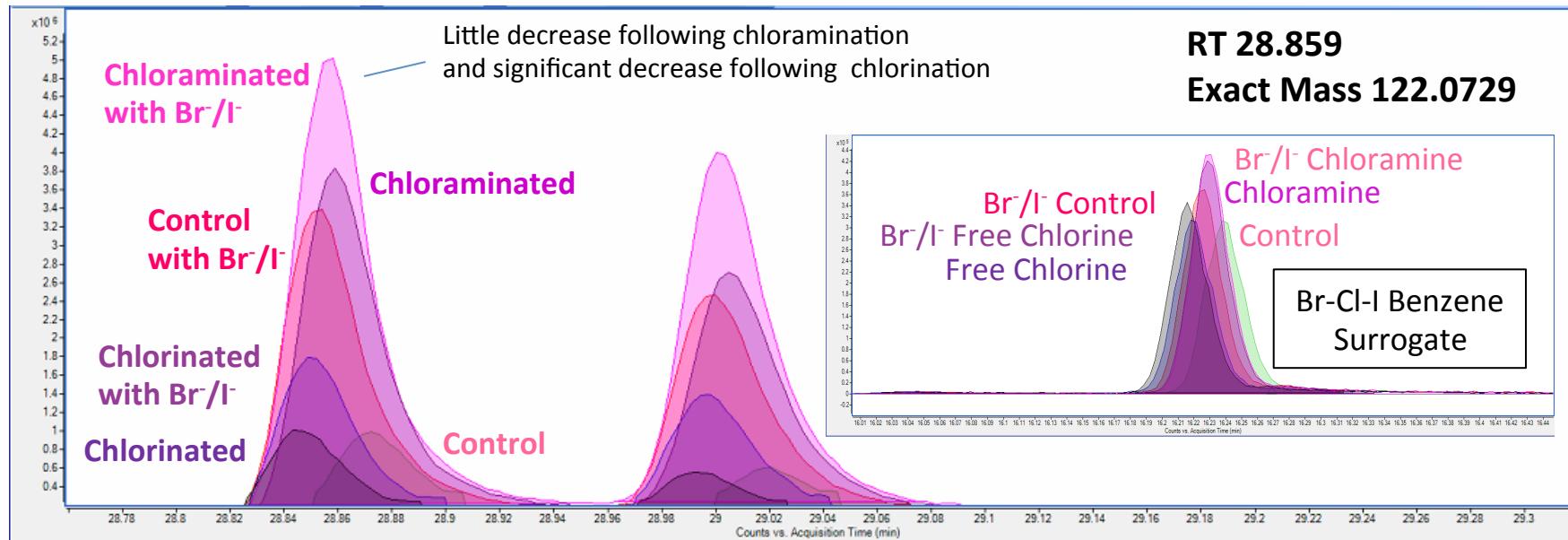


Figure. Shown above is the Agilent 7890B/ 7900 GC-ICPMS.

RT Locking with Br-Cl-I Benzene and F-I Benzene

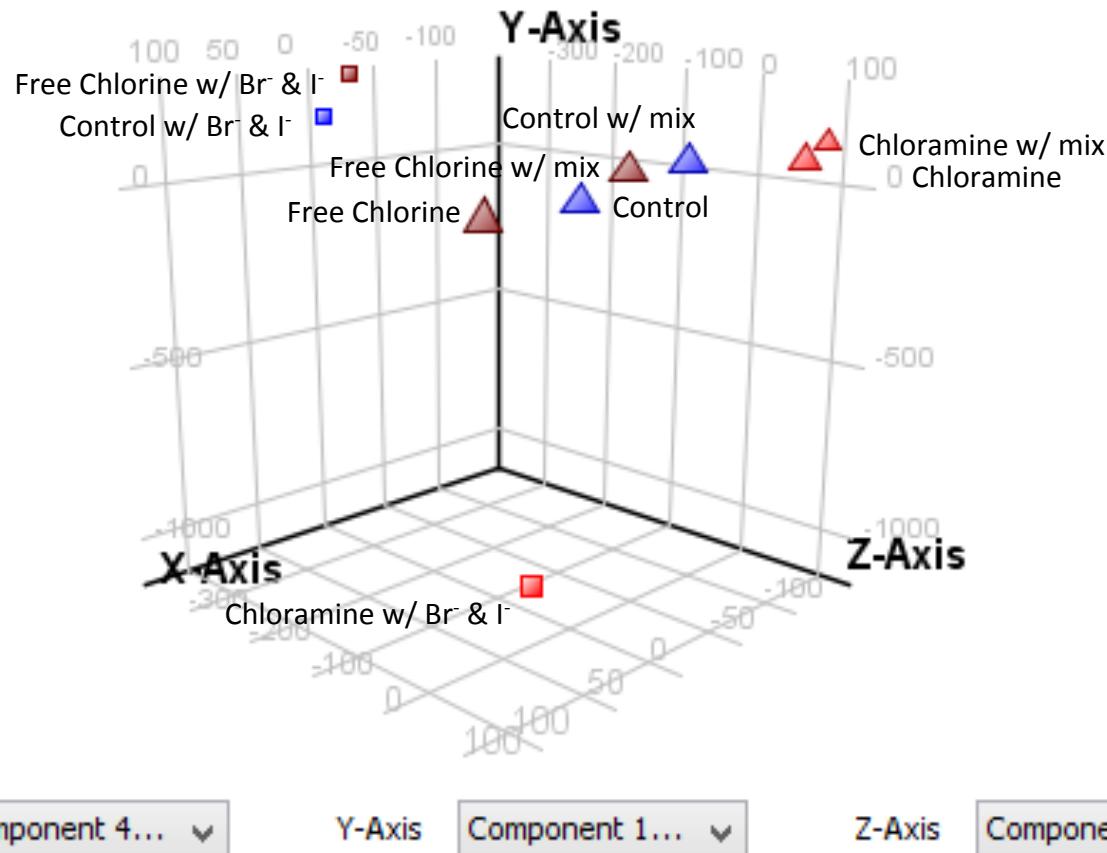


Overlay of Ion Chromatograms





PCA shows each sample as unique



The chloraminated samples in the presence of Br⁻ and I⁻ are most distant from the control.



Hierarchical Clustering

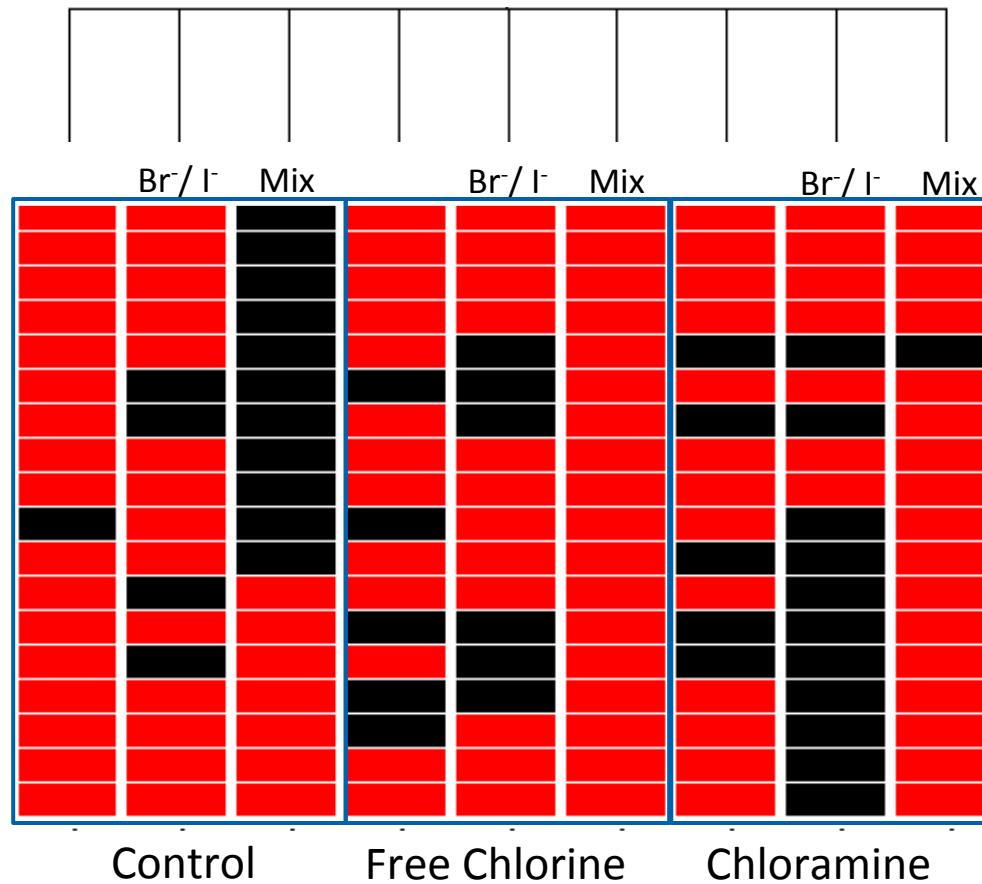
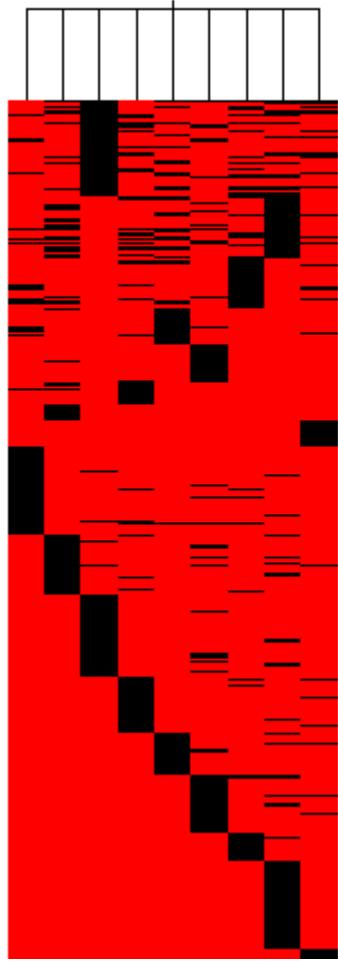
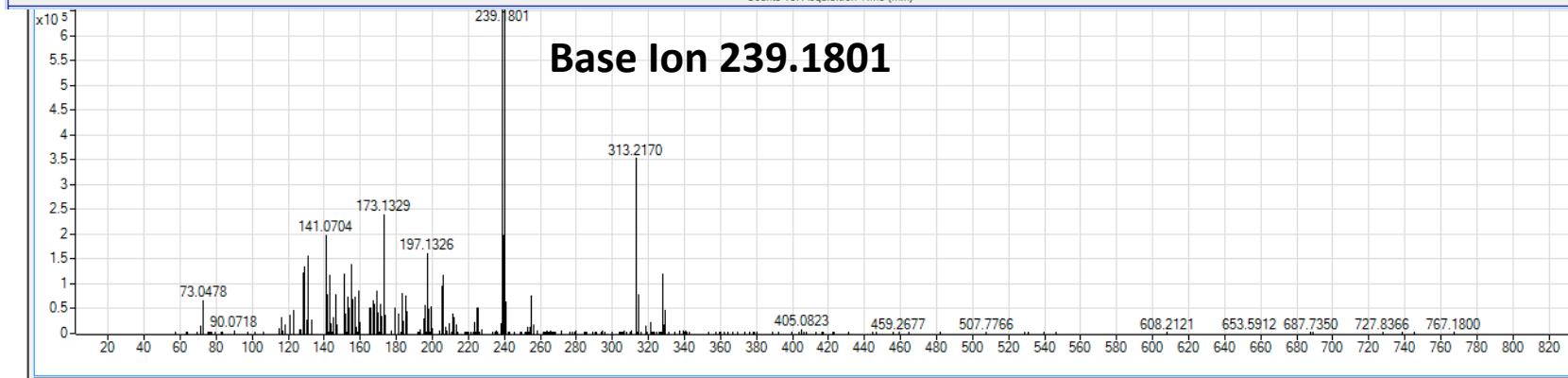
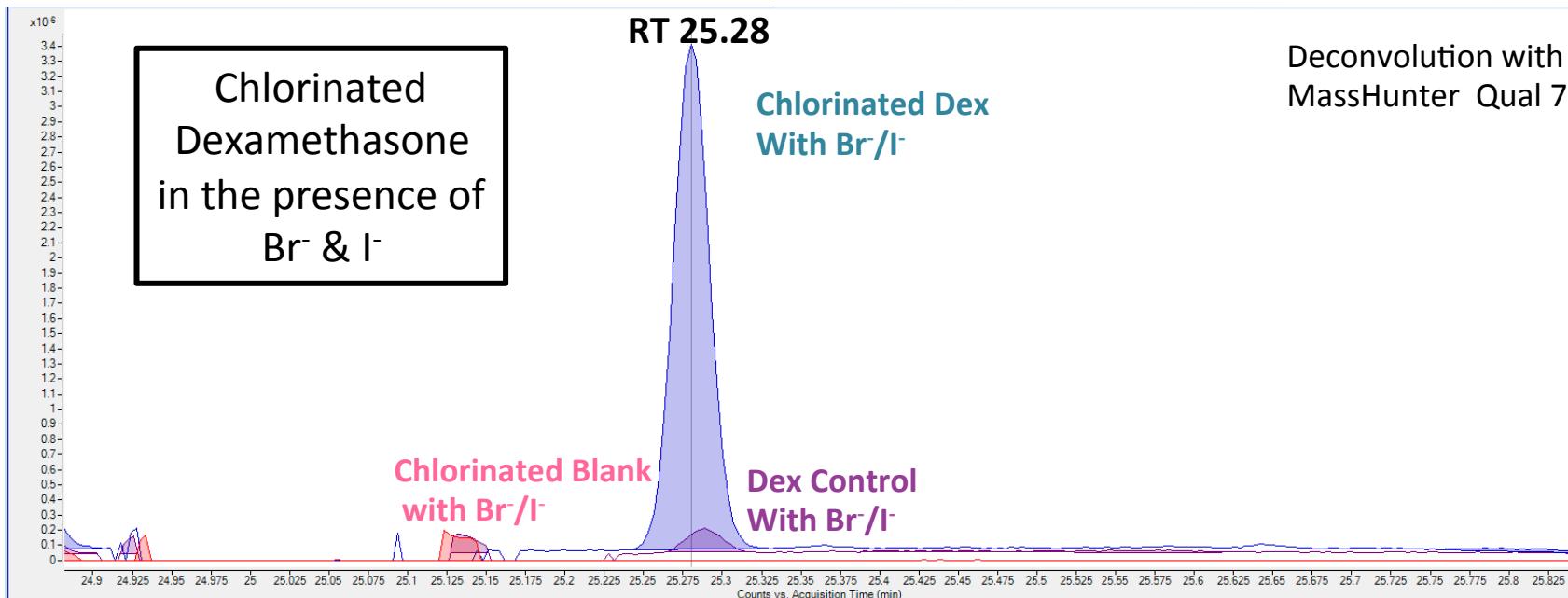


Figure. Comparison of all features present in treated Dex samples and control, and relative presence and absence.



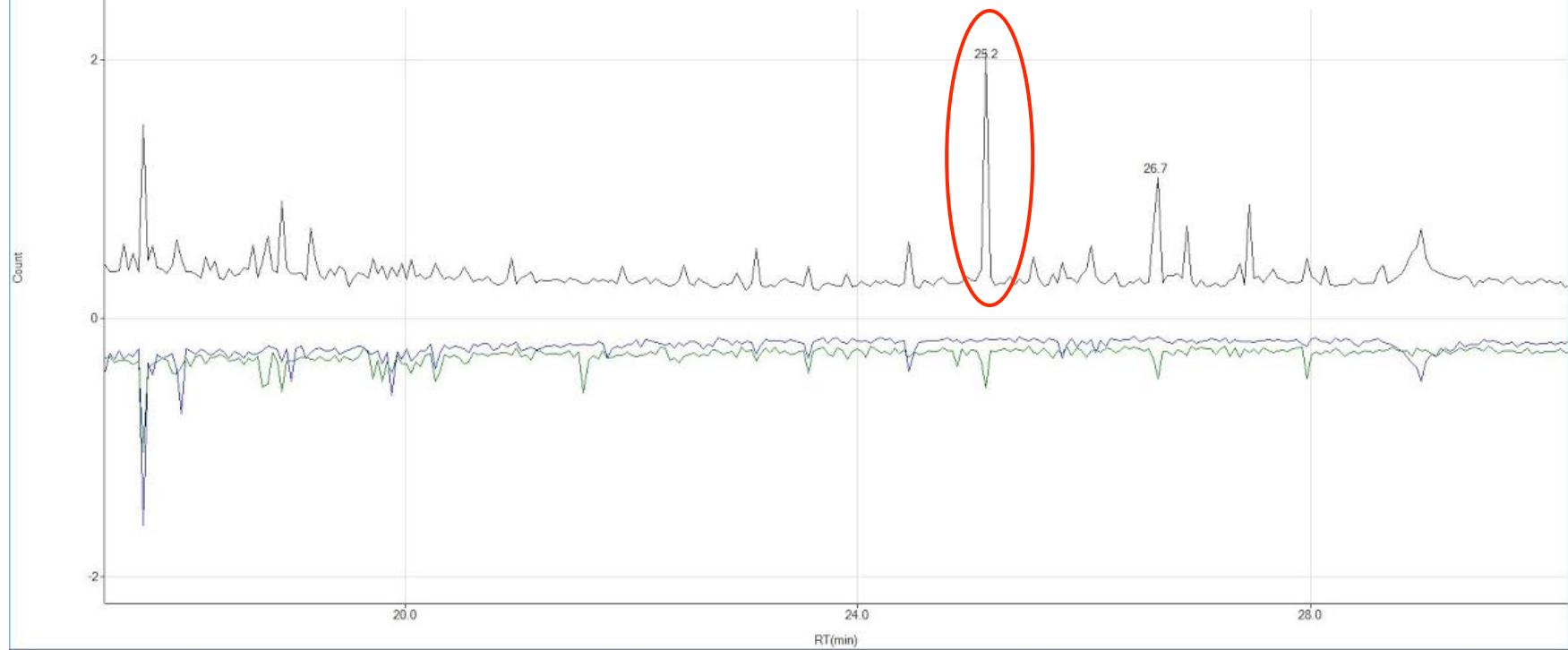
Brominated DBP





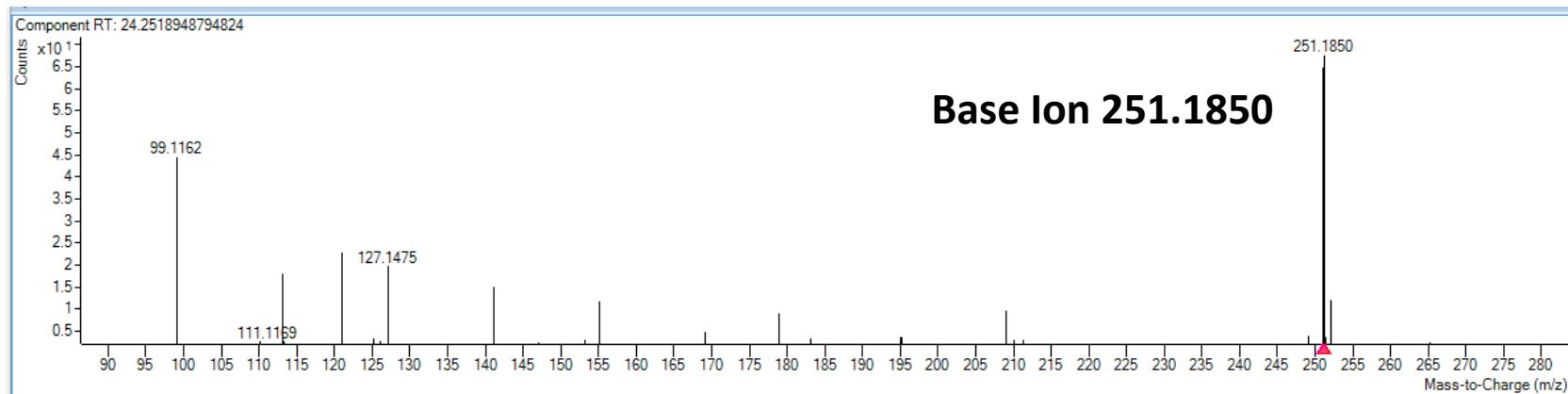
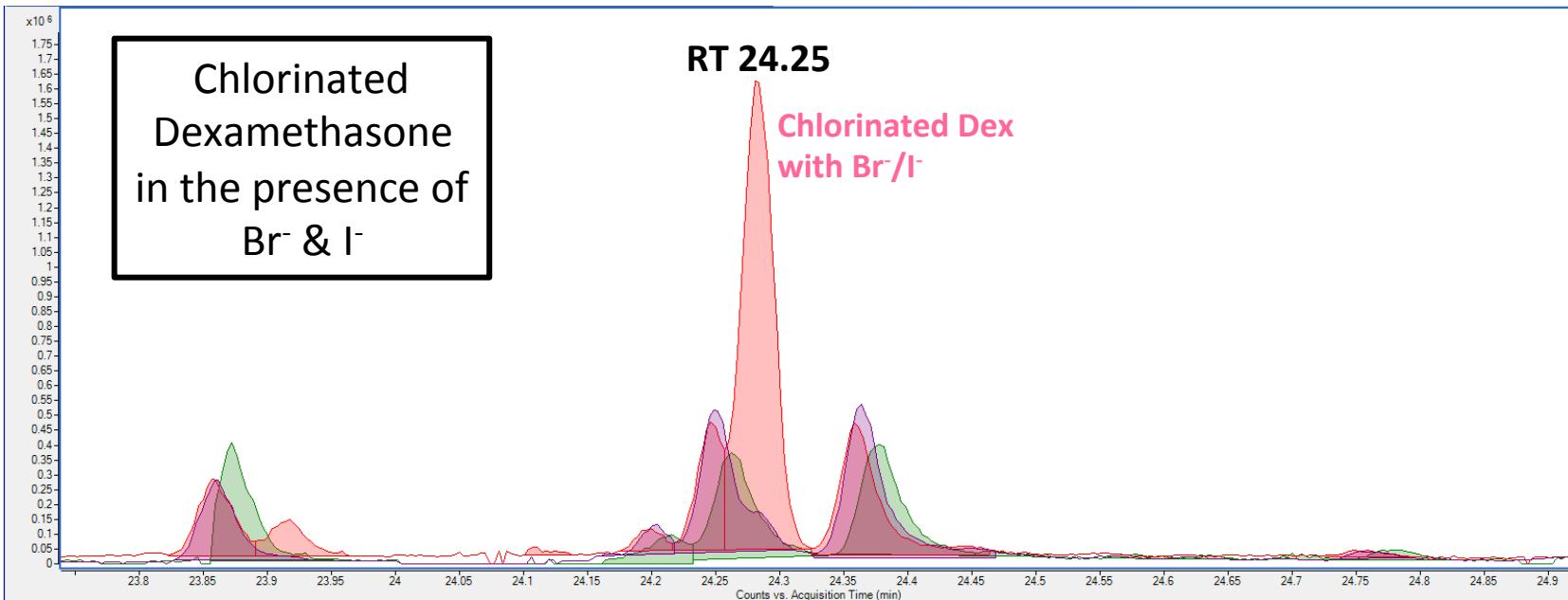
Br-79 Confirmation

x10³ Overlay of chlorinated Dex in the presence of Br⁻/I⁻ with chlorinated blank in the presence of Br⁻/I⁻ and Dex control in the presence of Br⁻/I⁻



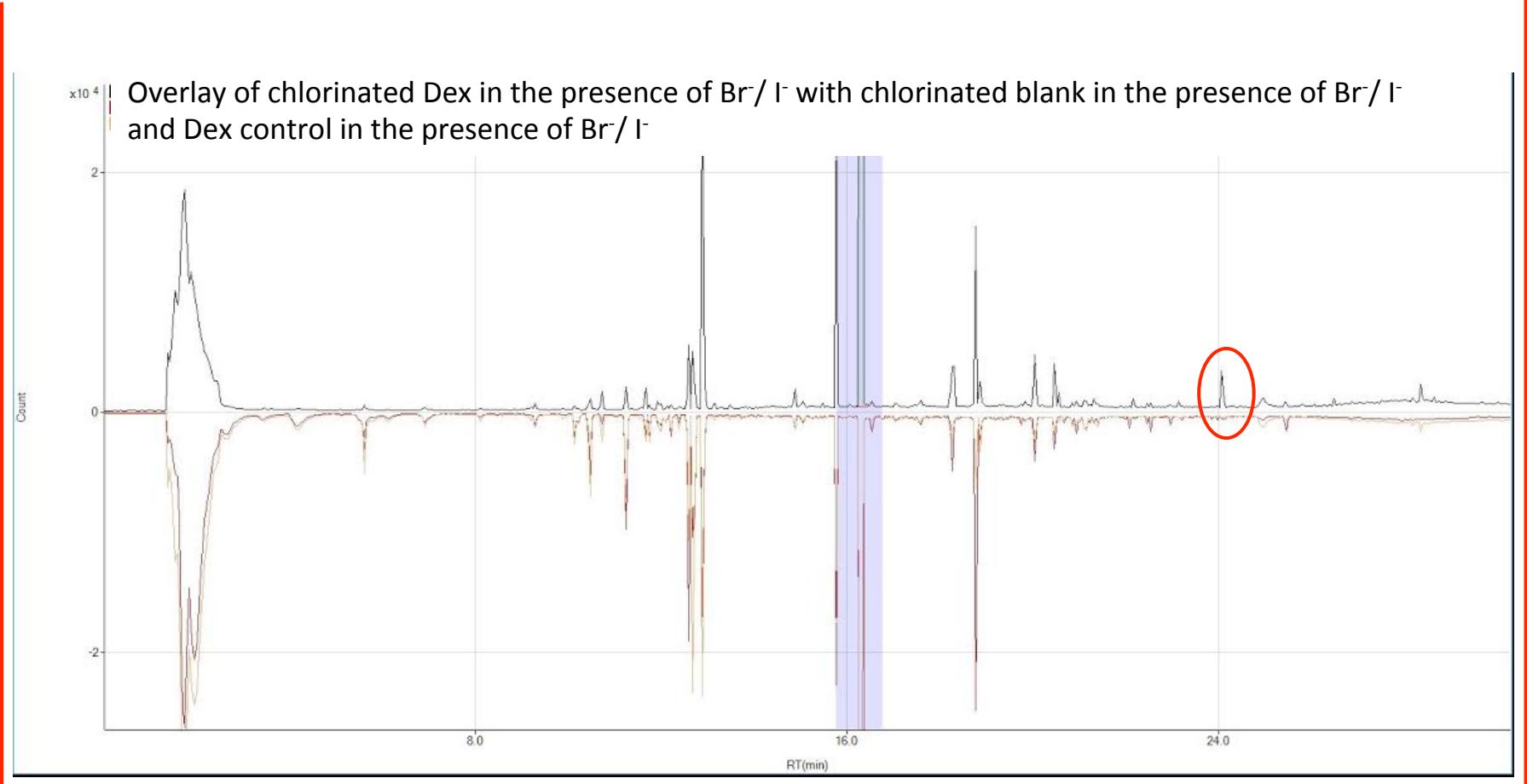


Iodinated DBP



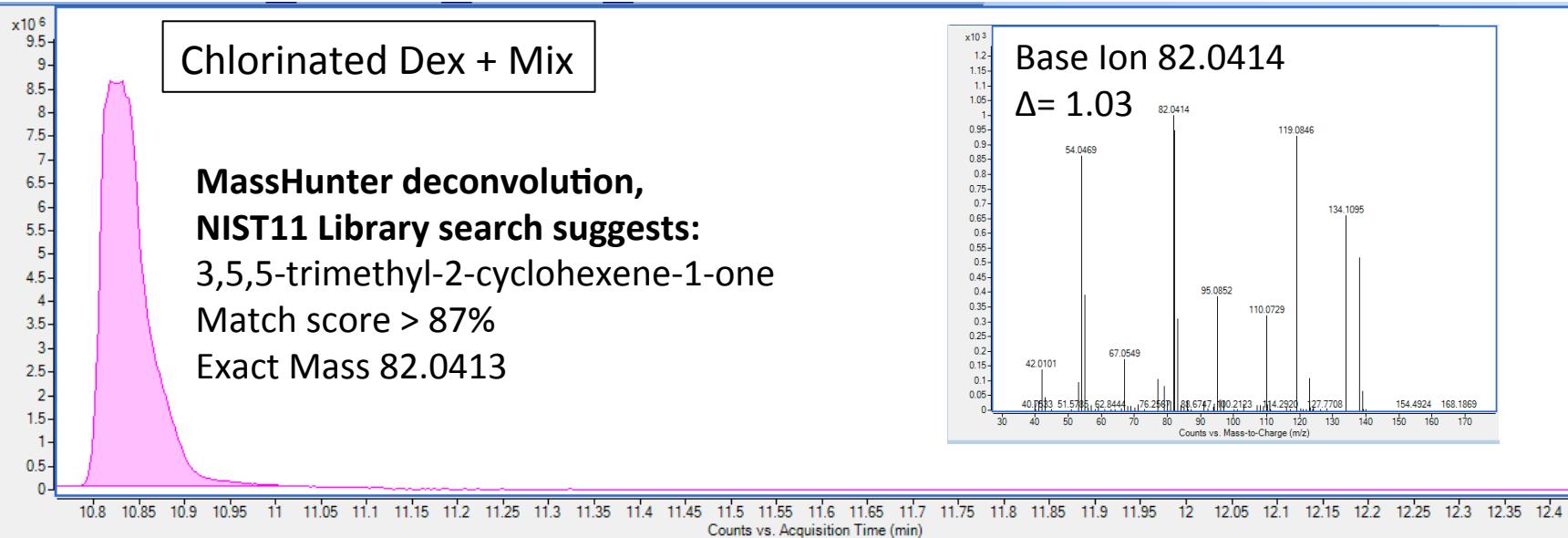


I-127 Confirmation

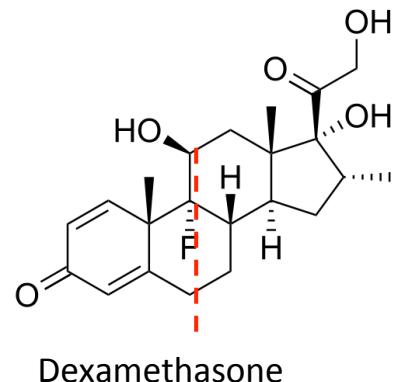




Putative ID of unknown at 10.82 minutes



**Proposed
Mechanism**





Summary

- Attenuation of Dexamethasone is greatest with free chlorine.
- Chlorination and chloramination of Dex formed a few halogenated DBPs.
- The presence of Br^- and I^- in water during disinfection seems to accelerate the oxidation process.
- Reduction in glucocorticoid receptor following oxidation of Dex. activity.
- Br^- and I^- during Dex oxidation leads to further reduction of GR activity.
- GR mixtures display antagonistic behavior against the GR receptor.



Future Direction

Further Analysis

Identify the untargeted compounds from the GC-QToF

Confirmation of compounds using CI mode

Identify the more polar DBPs using LC-QToF

Elucidate the identified DBPs from real water samples

Upcoming Research

Evaluate cytotoxicity and genotoxicity

Identify biomarkers of exposure with zebrafish embryo metabolomics



Acknowledgement



Agilent Technologies



Snyder Research Group

Prof. Shane Snyder

Dr. Armando Durazo

Dr. Christiane Hoppe-Jones

Kevin Daniels

Hao Vo



Questions

