

Novel Preparation and Analysis Method for Triclosan and Methyl Triclosan in Biosolids

Using Earthworms to Remove Contaminants from Biosolids

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Laboratory

*Research Project



- Biosolids/TAGRO program Division Manager at the City of Tacoma sponsored the research
- Investigate the efficacy of using earthworms to remove the antimicrobial (triclosan) and its transformational product (methyl triclosan) from biosolids
- Need Environmental Services Laboratory to provide analytical support for Whitney's research.

*Whitney P. Weibel

Evergreen State

College Masters

Degree Candidate

Vermiculture

Vermiculture -cultivating earthworms while transforming solid waste and organic biosolids into a soil amendment product.

Saves energy and water and reduces green house gas emissions.

Remediate certain organic and metal contaminants before they are applied to gardens, lawns or agricultural lands



Remediation

Earthworms biomagnify inorganic and organic contaminants as they consume and degrade organic matter in soils including...

- Mercury
- Lead
- Cadmium
- Polycyclic aromatic hydrocarbons
- Fire retardants
- Pesticides
- **Triclosan**



Triclosan

Personal care products

Hand soaps; detergents; shampoo & hair conditioners; skin creams; shaving creams; toothpaste; mouth washes; deodorants & antiperspirants; cosmetics; toiletries; laundry detergents & softeners; acne treatment products

Other household items

Fabrics; bedding; trash bags; paints; plastic cutting boards; kitchen utensils; dish washing products; carpet cushions; hot tubs, plastic lawn furniture; impregnated sponges; pesticides; computer equipments; polymers; textiles; coatings; ceramics; paper and adhesives

Health care

First aid; antiseptic products; surgical hand scrubs; antimicrobial agent; surgical gloves; preservatives; implantable medical devices

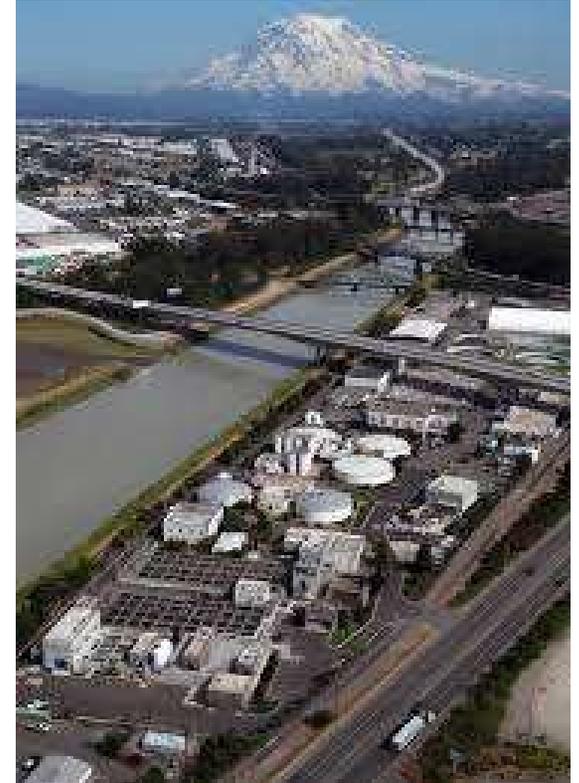
Sports items

Shoes; apparel (socks & undershirts); children toys

Triclosan: Current Status, Occurrence, Environmental Risks and Bioaccumulation Potential, *Int. J. Environ. Res. Public Health* 2015, 12(5), 5657–5684; Gurpreet Singh Dhillon , Surinder Kaur , Rama Pulicharla , Satinder Kaur Brar , Maximiliano Cledón , Mausam Verma and Rao Y. Surampalli

WWTP BIOSOLIDS

1. Incomplete Removal: Even after extensive plant processes and treatments much of the triclosan remains. Triclosan is very hydrophobic; it stays within the solids
2. Large Quantity: WWTP's generate over eight million dry tons of biosolids annually
3. Dispersed: 3/4 of these biosolids are used in agricultural lands and a 1/4 are used for gardening
4. Stable: Triclosan has a half-life of about 16 weeks, depending on multiple environmental conditions



Concerns about Triclosan

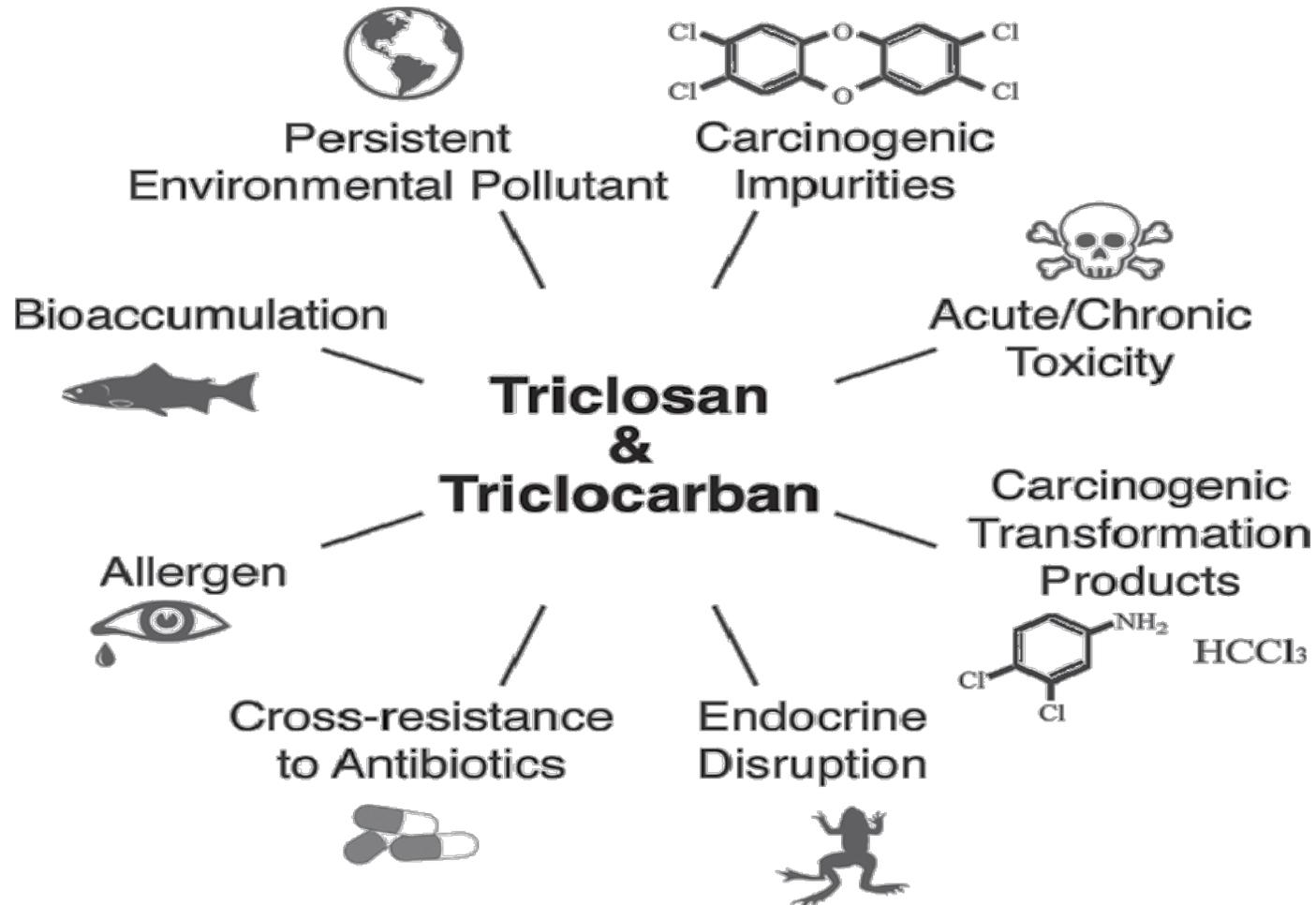
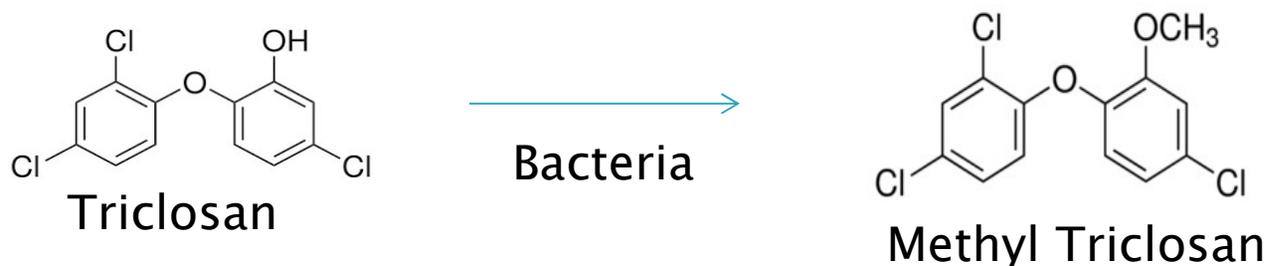


Image by Jason Diehl. On the Need and Speed of Regulating Triclosan and Triclocarban in the United States. Rolf U. Halden, *Environ. Sci. Technol.* 2014, 48, 3603–3611

Methyl Triclosan (MTS)



- ▶ Triclosan is a chlorinated aromatic compound with ether and phenol functional groups
- ▶ MTS is a metabolite of triclosan (bacteria) that is also present in biosolids
- ▶ Even more hydrophobic, bioaccumulative and stable
- ▶ Not as well studied, but assumed to have similar environmental effects as triclosan

Project Goals

Overall
Goals

Preparation

Extraction

Analysis

HIGH QUALITY DATA

CONSTRAIN COST,
TIME AND EFFORT

SIMPLE EXTRACTION

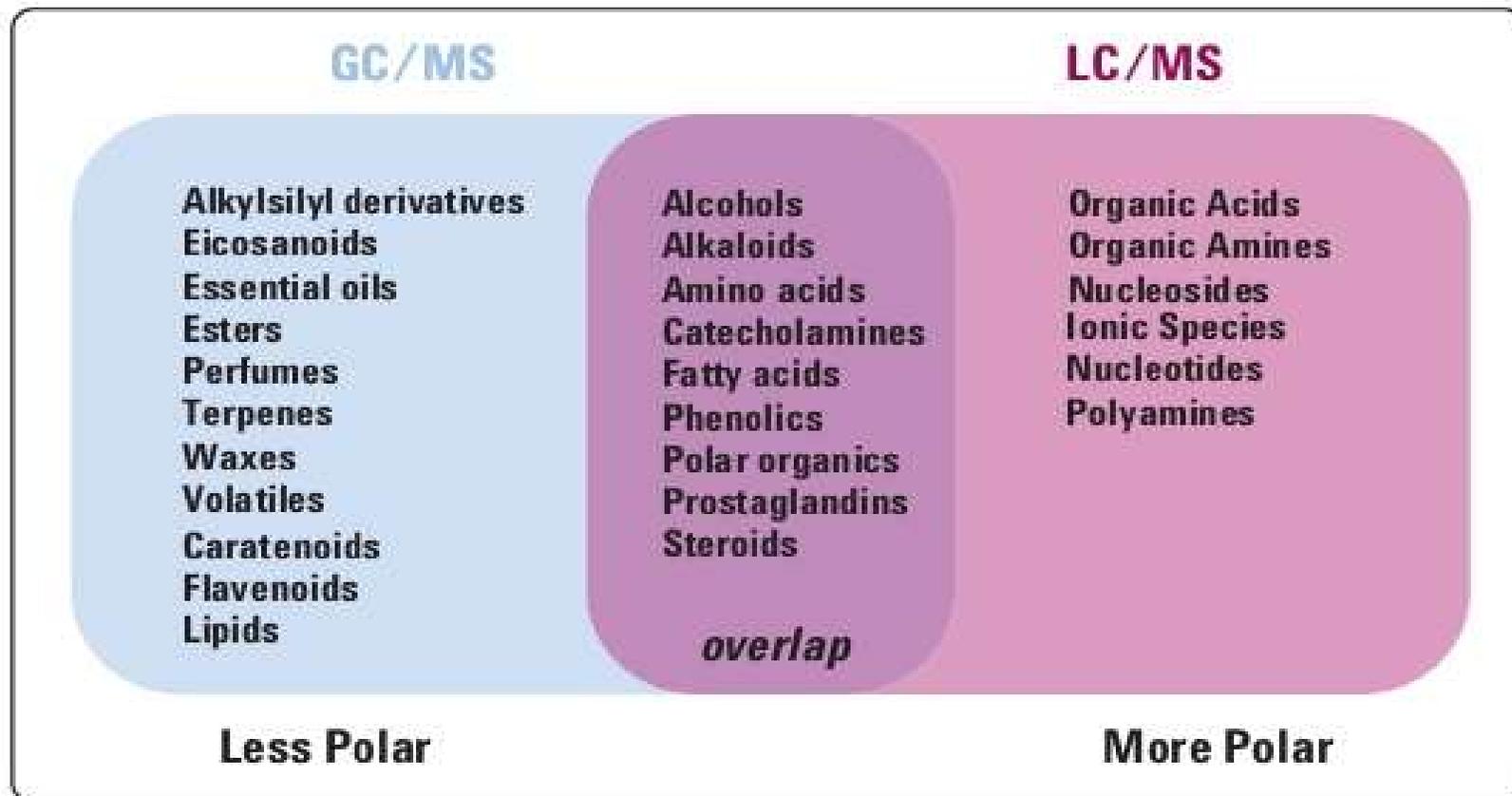
NO CLEAN UPS

NO SOLVENT EXCHANGE

NO DERIVITIZATION STEPS

ONE INSTRUMENT

GC-MS vs. LC-MS



What about the LC/QQQ?

Triclosan

- Had an existing method
- Good sensitivity

Methyl Triclosan

- Could not find a method in literature
- Had problems with my initial effort
- Aggressive time schedule for developing the method
- Shared instrument



What about the GC/MS or GC/QQQ?

Triclosan

Poor sensitivity

Must be derivitized

(for compounds that have poor volatility or decompose easily with heat)

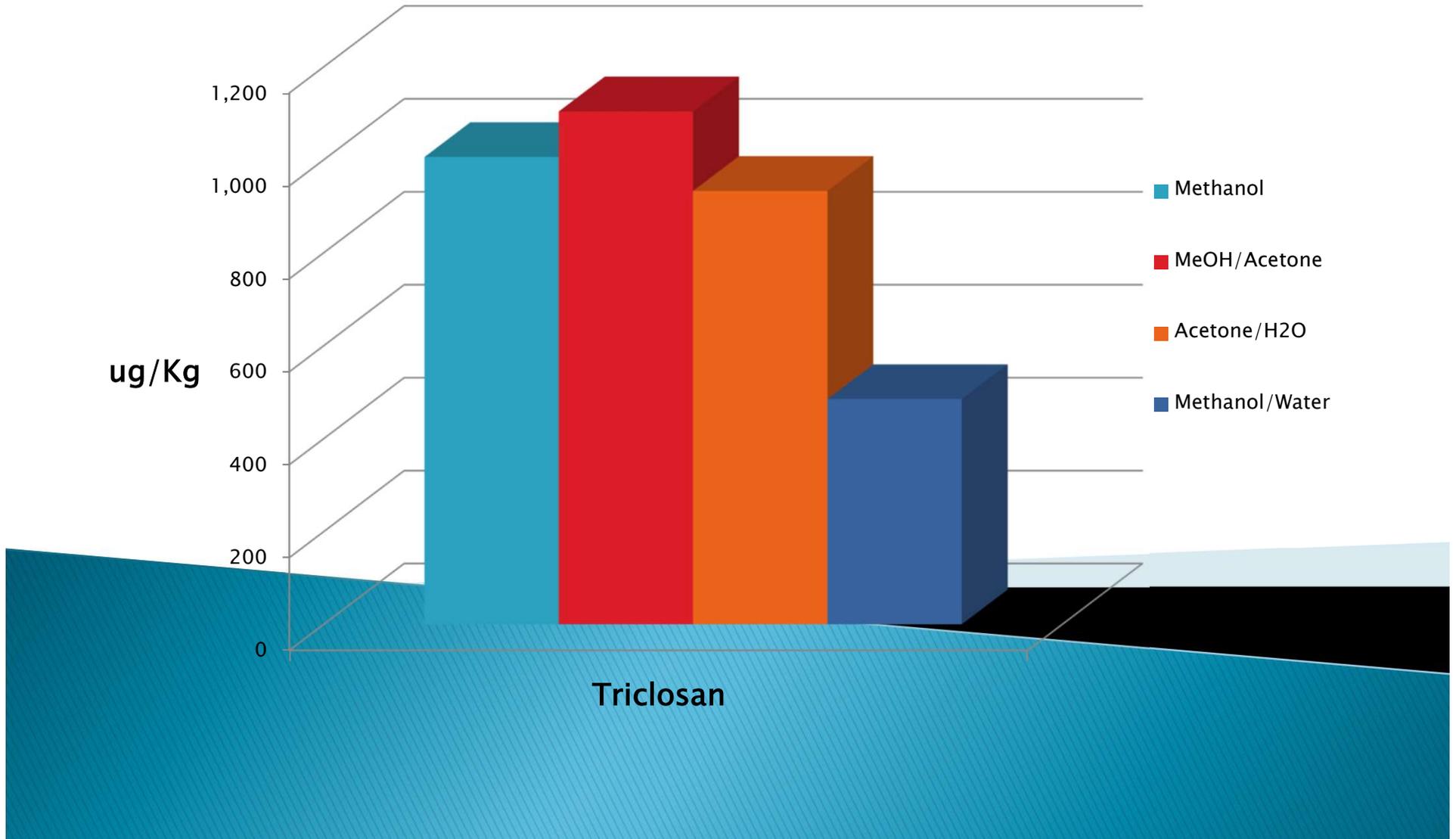
Produce methyl triclosan

Methyl Triclosan

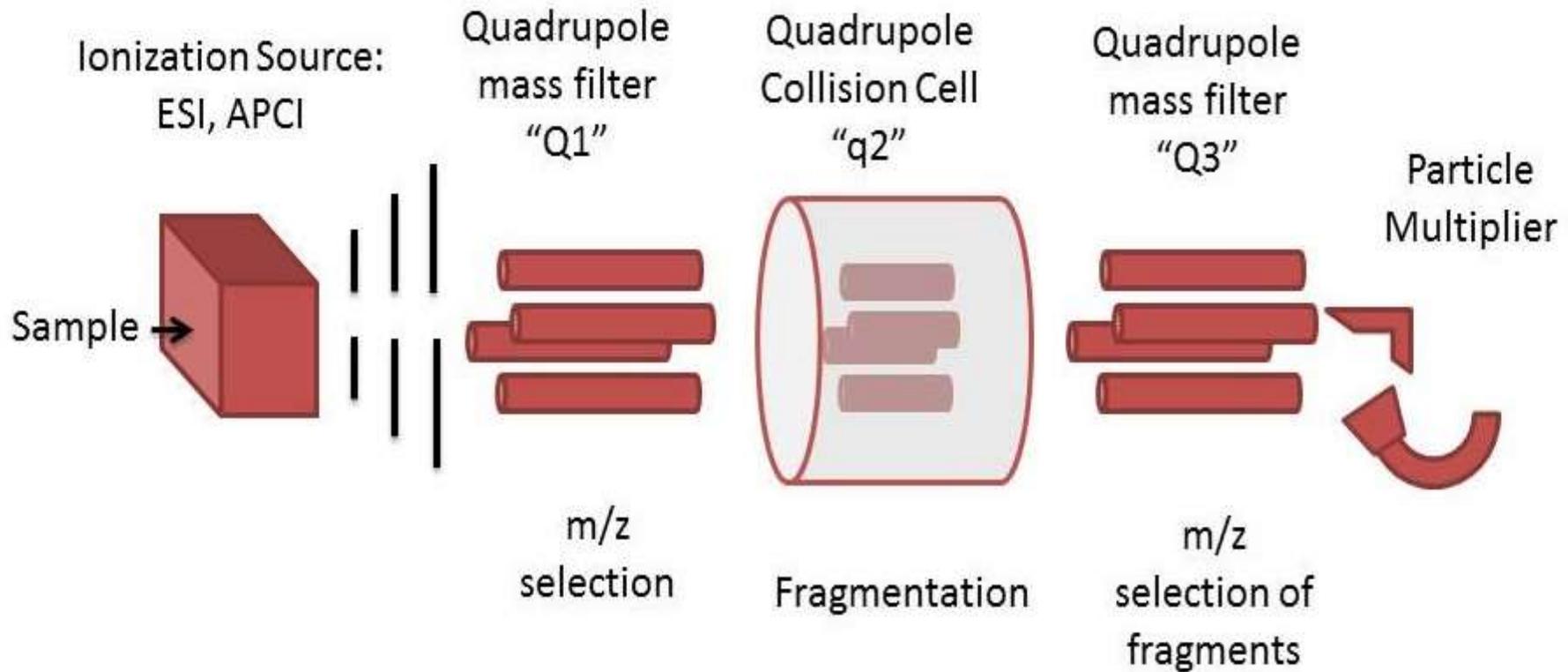
Good sensitivity



Triclosan in Cake – LC/QQQ

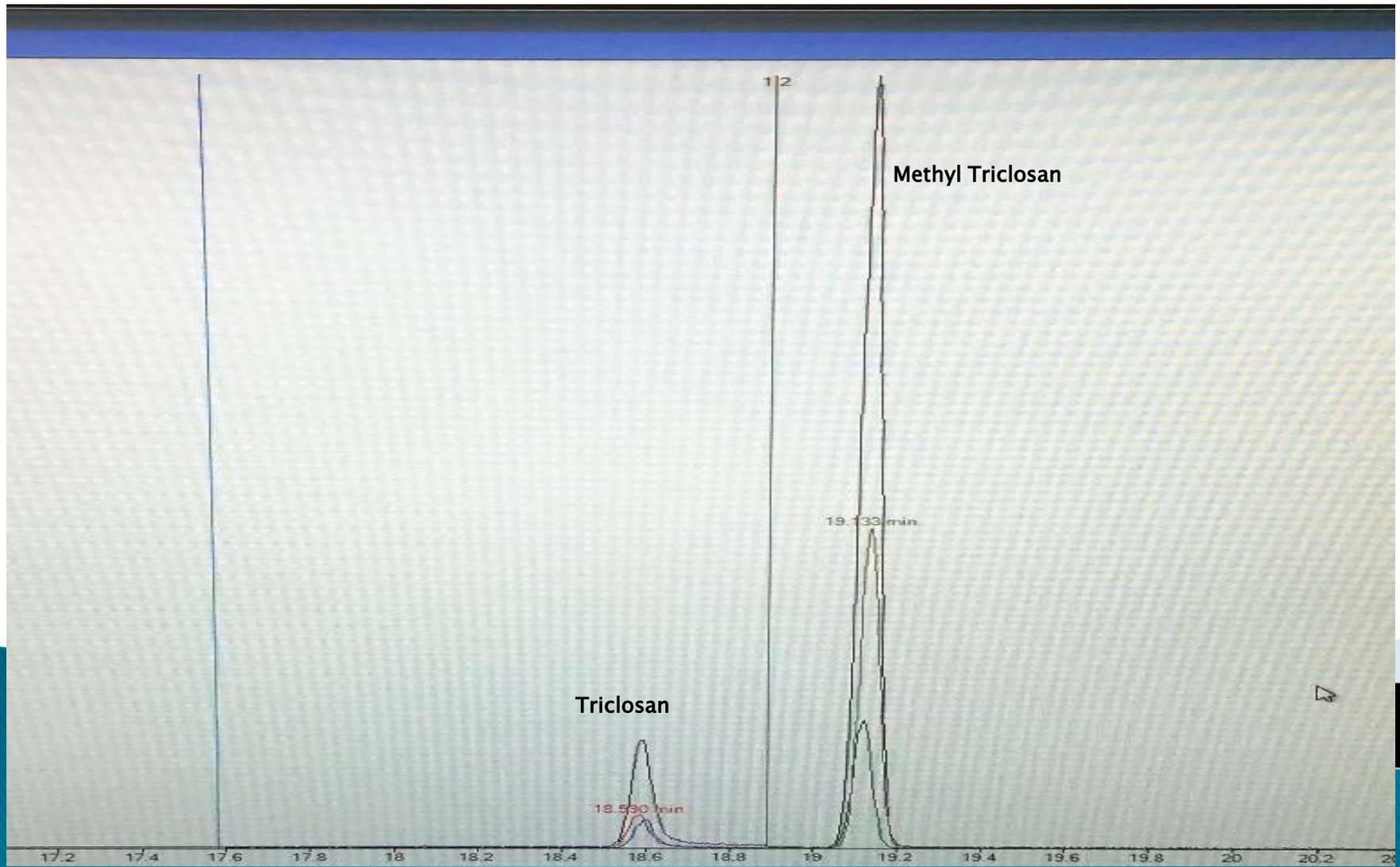


Decided on GC/QQQ for Specificity and Matrix Interference

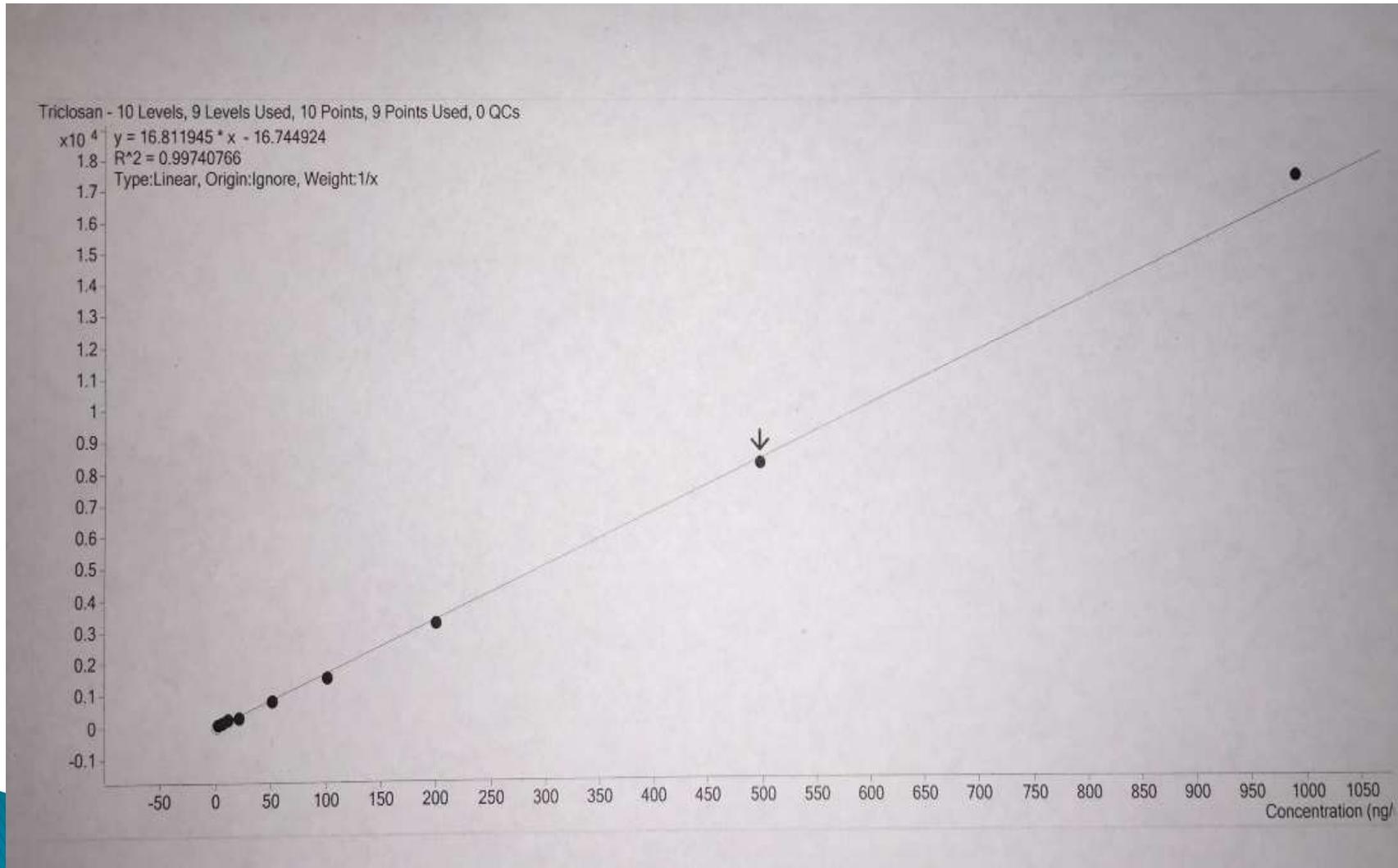


Brian Shuhler, "Analysis of the organochlorine Pesticide Routine Target List by Gas Chromatography/Tandem Mass Spectrometry" US EPA Region 4

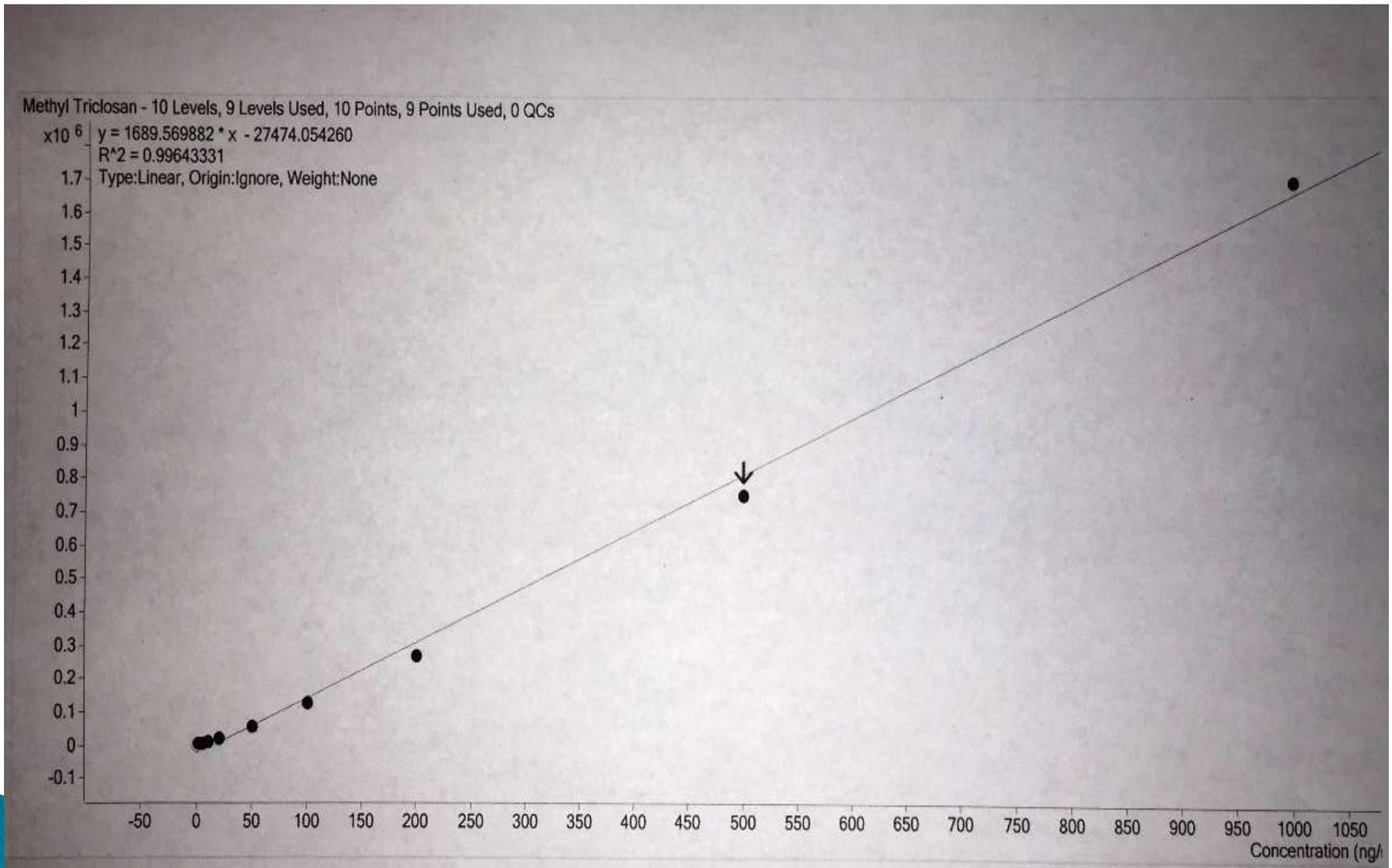
Chromatography



Calibration for Triclosan



Calibration for Methyl Triclosan



Preparation and Extraction



Mill

Methylene Chloride

Vortex



Sonicate



Filter



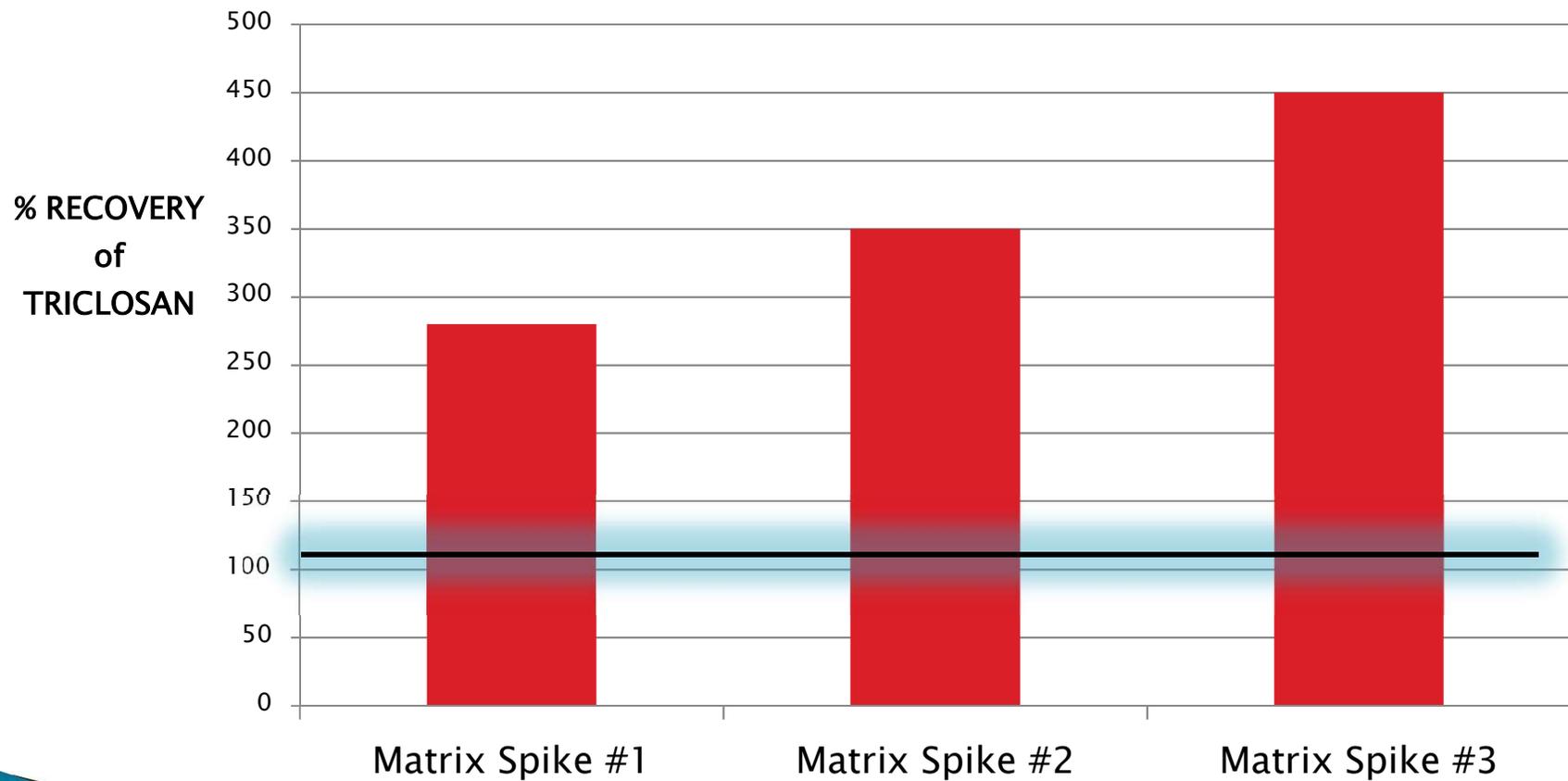
Triclosan/MTS Initial Results

GC/QQQ successfully cut through the biosolids matrix!

- ▶ Triclosan around 4–5 mg/kg (ppm)
 - Within literature values
 - Higher than extraction amount on LC/QQQ using methanol/acetone 2.2 mg/kg
- ▶ Methyl Triclosan – near detection level
 - MTS is produced in an aerobic environment
 - City of Tacoma has a dual aerobic/anaerobic treatment process



Increasing Sensitivity with Repeated Injections?



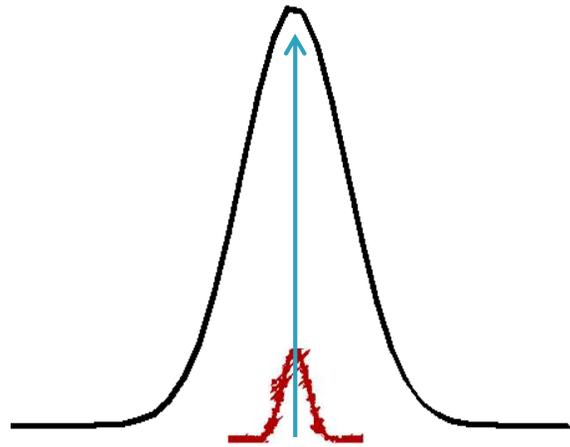
What is going on?



Source of High Matrix Spike Recoveries

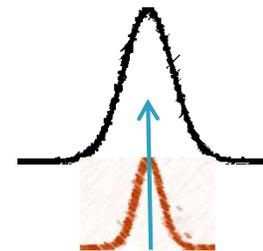
Worm Bin Sample Consists of
Two Components

1. Paper Mulch



Large Enhancement

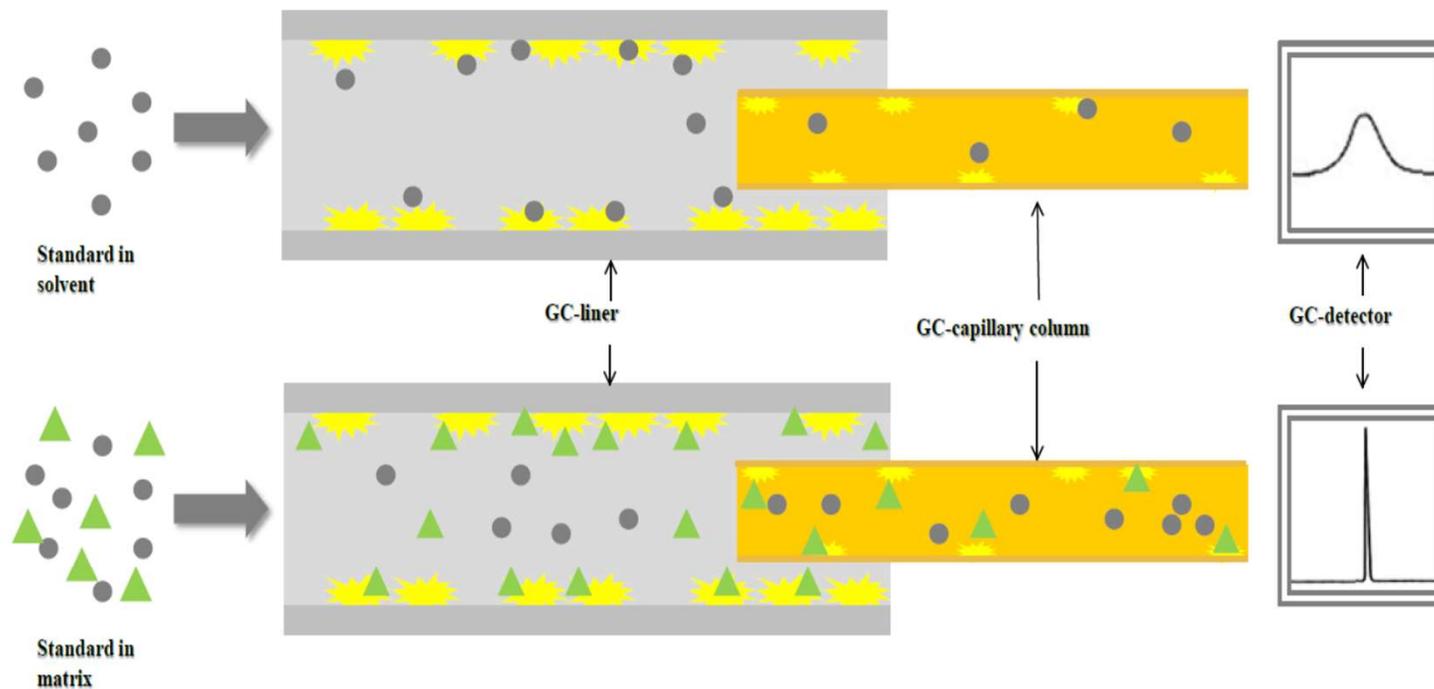
2. Biosolids



Small Enhancement



Introduction



- ★ Active site (silanols, metal ions, and other active sites)
- Target analytes
- ▲ Matrix components

Matrix enhancement effect: a blessing or a curse for gas chromatography?--A review. [Anal Chim Acta](#). 2013 Nov 1;801:14-21. doi: 10.1016/j.aca.2013.09.005. Epub 2013 Sep 13. [Rahman MM](#), [Abd El-Aty AM](#), [Shim JH](#).

Sensitize Instrument

- ▶ Tried to sensitize instrument before the analytical run with repeated injections until sensitivity stabilized
- ▶ Did not work!
- ▶ Still unacceptable reproducibility/stability.



Matrix Enhancement Solutions

Extensive Cleanup –

But our goal was a simple procedure

Standard Addition – differing amount of standard is added directly to aliquots of analyzed sample

Matrix Matched Standards –

Provide the same amount of matrix-induced enhancement in calibration standards as the sample extracts



Take Advantage of the Matrix Effect!

- Again, the major issue of analyzing underivatized triclosan was its low response on the GC/QQQ
- Since the matrix effect provides larger and higher quality peaks, **why not take advantage of it, instead of removing it?**



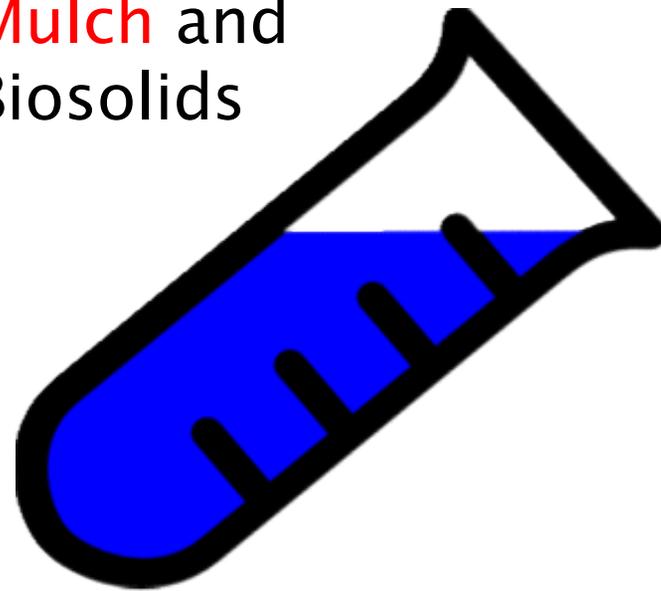
Matrix Matching

Paper
Mulch



Calibration
Standards

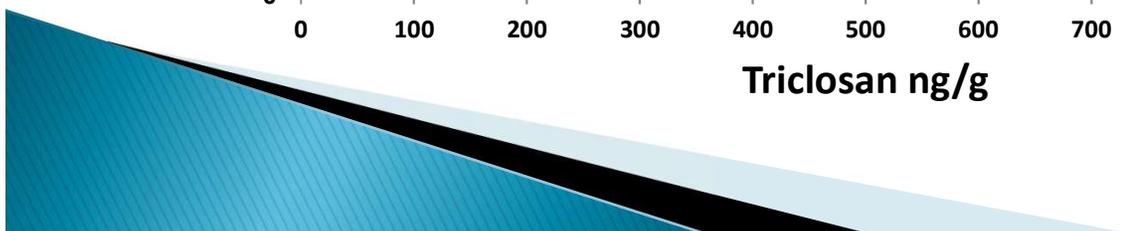
Paper
Mulch and
Biosolids



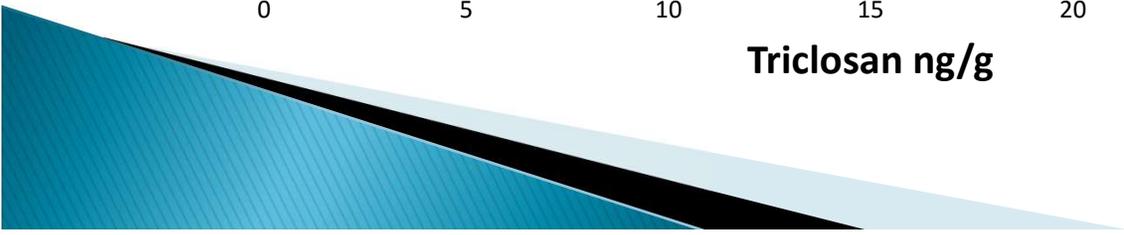
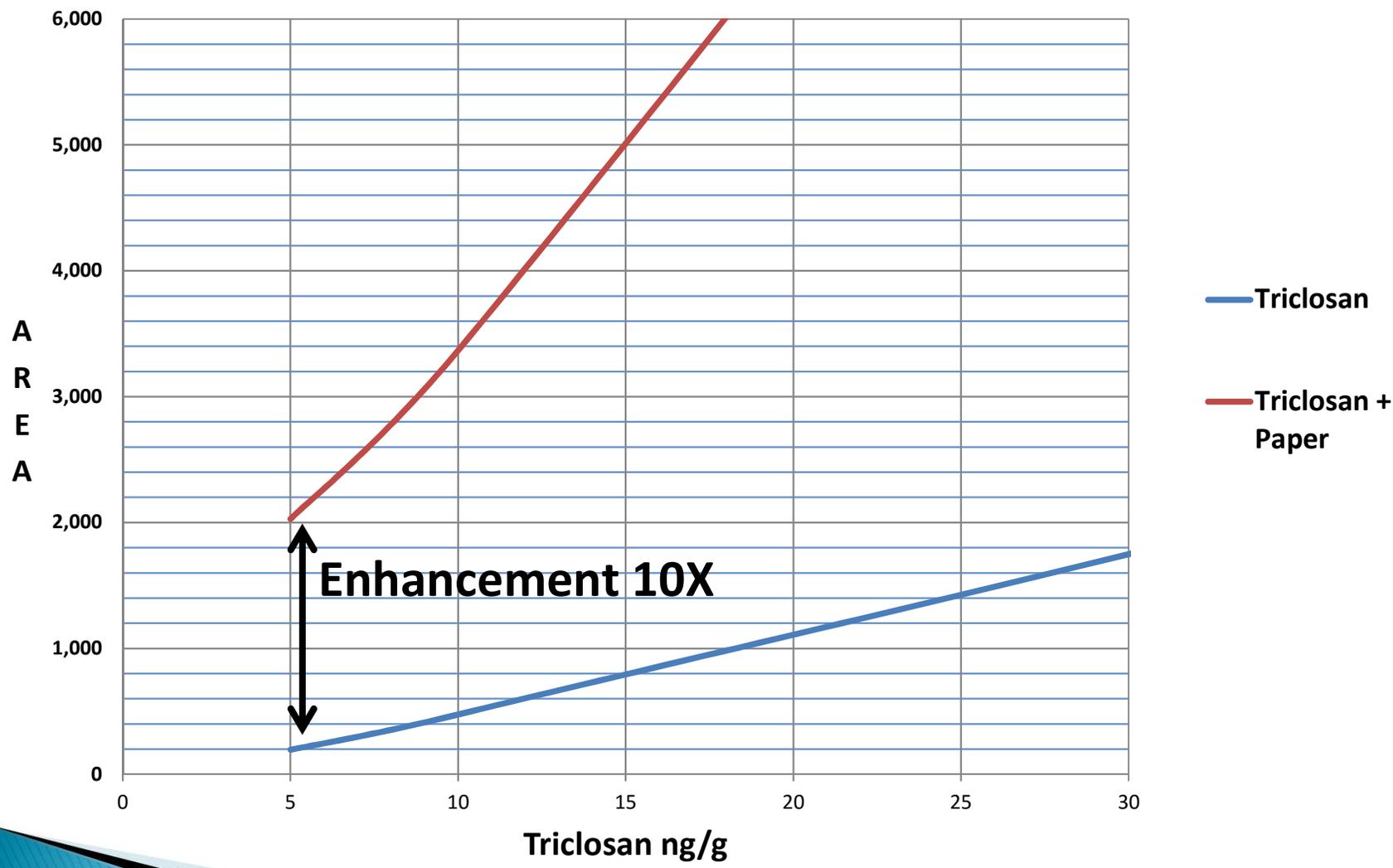
Samples



Calibration with Paper Mulch VS No Matrix



Calibration with Paper Mulch VS No Matrix



Sensitivity Increased But...

- ▶ Lower levels of quantitation was achieved for triclosan due to the enhancement
- ▶ Sensitivity also increased approximately 50% for methyl triclosan
- ▶ However, samples still had a lot of variability!!!

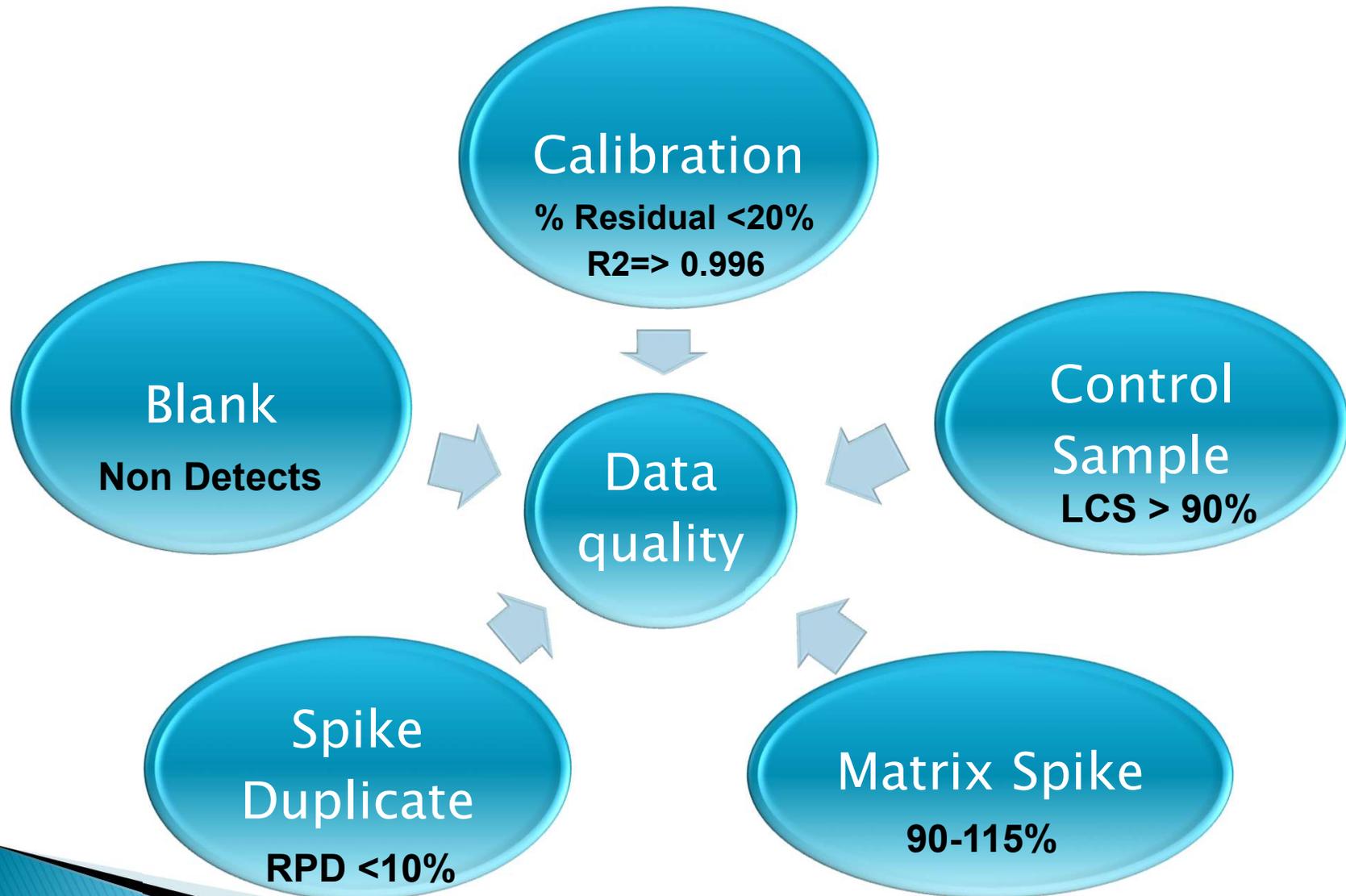


Isotopic Dilution May Be Solution

- ▶ Isotopic analysis uses an isotope variation of the analyte being measured as the internal standard. C-13 Triclosan and C-13 Methyl Triclosan
- ▶ Since they have different masses they can separately be measured on a mass spec
- ▶ And since they are otherwise exactly the same compound, they should behave the same way (suppression, enhancement, extraction efficiency).



Results Using Method



***So, using a simple extraction
and a novel analysis method,
excellent results can be
achieved for triclosan and
methyl triclosan within the
complex matrix of biosolids!***



Highlights

- ▶ Fast and easy methylene chloride extraction using vortex and sonication of milled samples
 - ▶ No sample clean ups, derivitizations, or solvent transfer
 - ▶ GC/QQQ analysis involving matrix matched calibration standards and isotopic dilution for enhanced sensitivity, selectivity and stability when performing analyses within the complex matrix of biosolids.
 - ▶ Novel use of newspaper extract to greatly enhance the sensitivity of triclosan
- 

Method Detection Level of Triclosan Comparison

| | AXYS Analytical Service LC/QQQ | Method GC/QQQ |
|------------------------------|-----------------------------------|------------------|
| Detection level Biosolids | 120 ng/g | 20 ng/g |



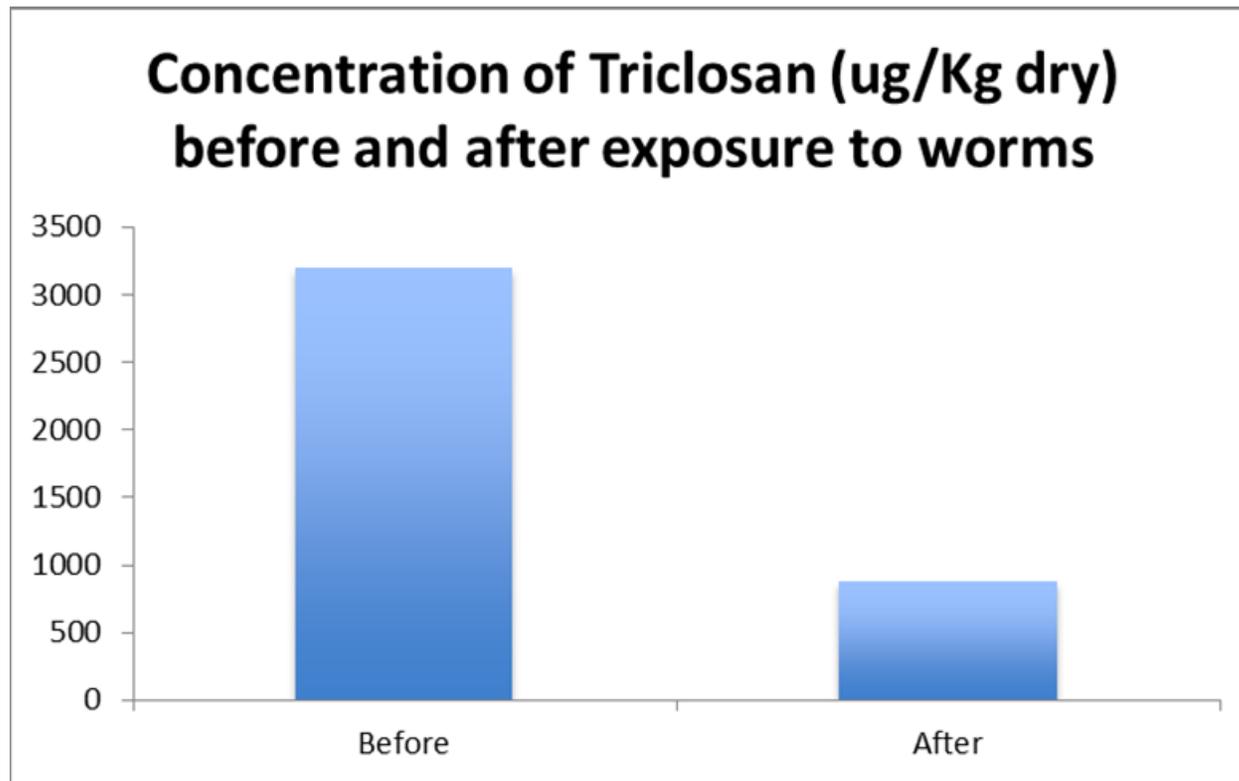
Calibration Comparison

| Analyte | Calibration Range ng/g (ppb) | Calibration Range ng/g (ppb) With paper mulch | Correlation R2 | Residuals |
|------------------|------------------------------|--|----------------|-----------|
| Triclosan | 10 – 1000 | 1 – 1000 | >0.995 | <20% |
| Methyl Triclosan | 1 – 200 | 0.5 – 200 | >0.995 | <20% |

Earthworm Removal Results

Eisenia fetida

Pilot Study



**Removed 73%
of the
triclosan in 4
weeks!**

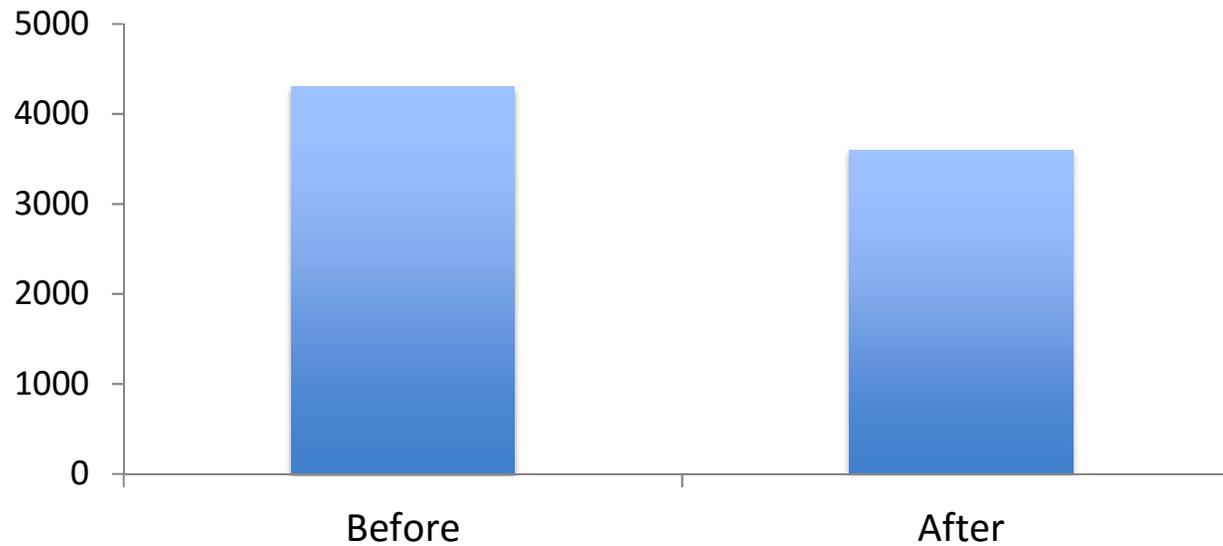
Very high mortality!

Earthworm Removal Results

Eisenia fetida

Study

Concentration of Triclosan (ug/Kg dry)
before and after exposure to worms



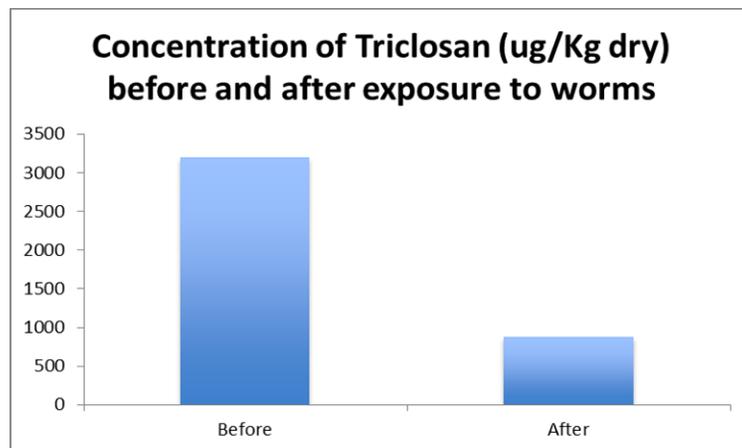
**Little or no
removal of
triclosan**

No mortality!

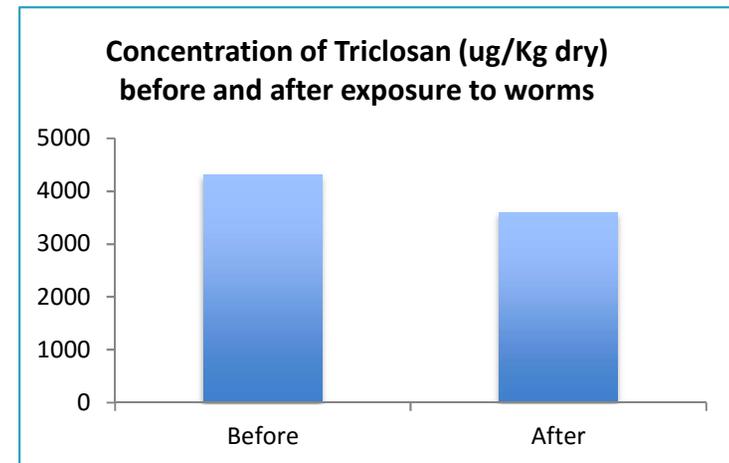


Possible Explanation of Difference

Juvenile Earthworms



Adult Earthworms



▶ **Age?**

▶ **Growth?**

▶ **Toxicity?**

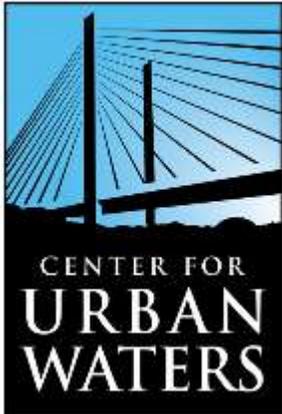
▶ **Ammonia - Longer aeration of cake**

Methyl Triclosan

- ▶ Tacoma's biosolids were just above detection, so the magnitude of change was slight
- ▶ Controls with no worms showed a slight increase in MTS levels
- ▶ Not definitive



Questions



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Center For Urban Waters, Tacoma WA

What is in the Recycled Newsprint that Creates a Matrix Enhancement?

- ▶ Paper Extractables - Waxes, fats, resins, terpenes, sterols, lignins
- ▶ Biosolids Extractables - Diols such as glycols are in large quantities

