

## Introduction

Increasing pollution has caused great concern regarding human exposure. Stationary sampling systems are limited in number/location and could over or underestimate personal exposure. Silicone wristbands have shown great ability to capture semi-volatile organic compounds (SVOCs) (Donald *et al.* 2016) and have the potential to have a great impact on increasing knowledge of personal human exposure. Non-targeted analysis (NTA) methods monitor emerging contaminants with potential for human exposure by qualitatively identifying chemicals and estimating concentrations on a greater chemical space scale than targeted methods. Silicone wristbands have been used for suspect screening and NTA on LC systems, but a standardized and tested procedure for GC and GC-HRMS has not been established. Here we begin developing a non-targeted analysis method for GC-HRMS applications by use of silicone wristbands.

## Methods

Many procedures exist for both cleaning silicone wristbands in preparation for deployment and extraction of analytes. Cleaning and extraction methods for silicone wristbands were tested for capture of the most analytes with the least amount of interference.

### Cleaning Methods

#### 1. Vacuum Oven

- 3 h @ 300 °C (Anderson *et al.* 2017)

#### 2. Soxhlet Extraction (Kile *et al.* 2016)

- 3 Extractions at ~20 cycles/h for 12 h
- Extraction Solvents used were:
  - Hexane
  - First ethyl acetate
  - Second ethyl acetate
- Wristbands were then soaked in methanol overnight
- In between each solvent change, samples were dried in a vacuum oven for 4 h @ 40 °C

### Extraction Methods

#### 1. Accelerated Solvent Extraction (ASE)

- 1500 psi at 80 °C for 5 min static cycles (x3). Flush volume at 60%
- Total of 108 mL combined extracts

#### 2. Soxhlet Extraction

- Two ethyl acetate extractions
- Total of 300 mL combined extracts

#### 3. Sonication

- 50 mL ethyl acetate for 15 min (x3)
- Total 150 mL combined extracts

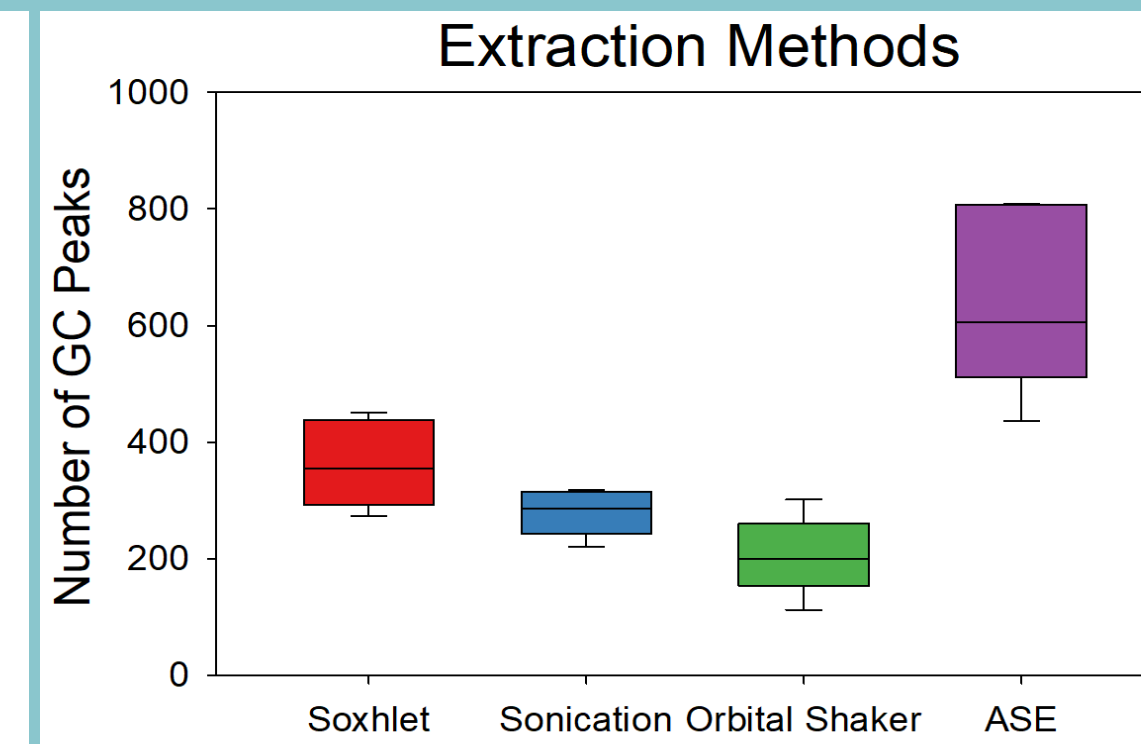
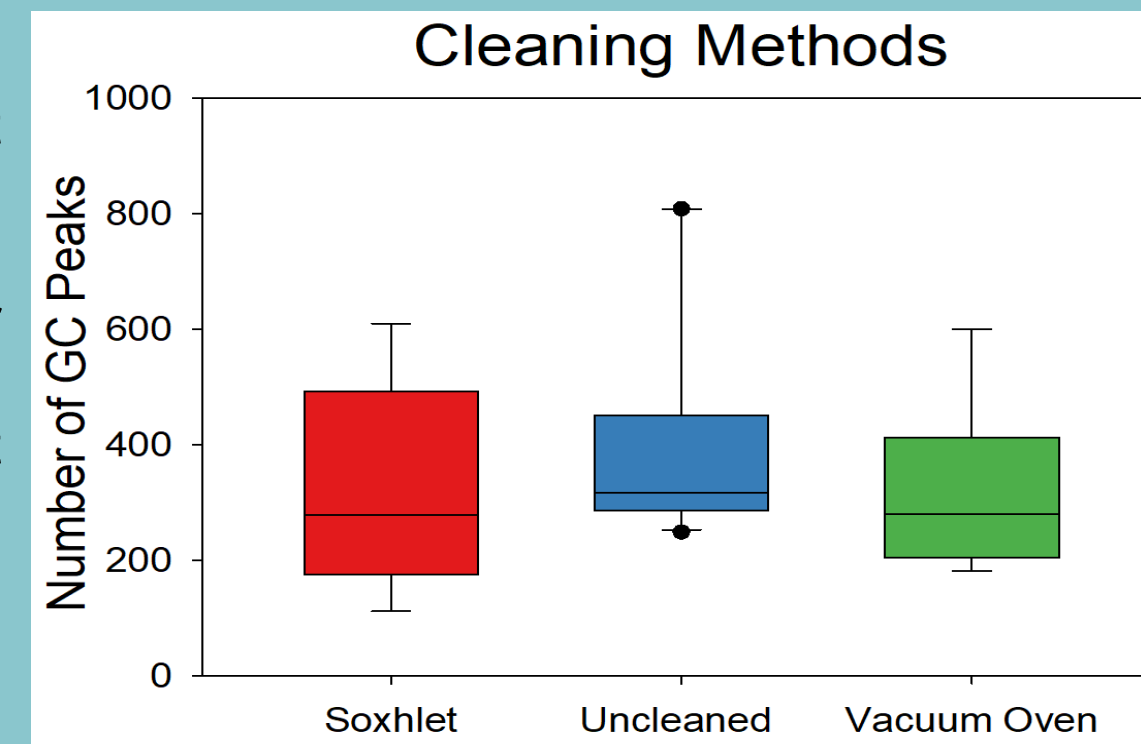
#### 4. Orbital Shaker

- 100 mL ethyl acetate @100 RPM for 2 h (x2)
- Total of 200 mL combined extracts.

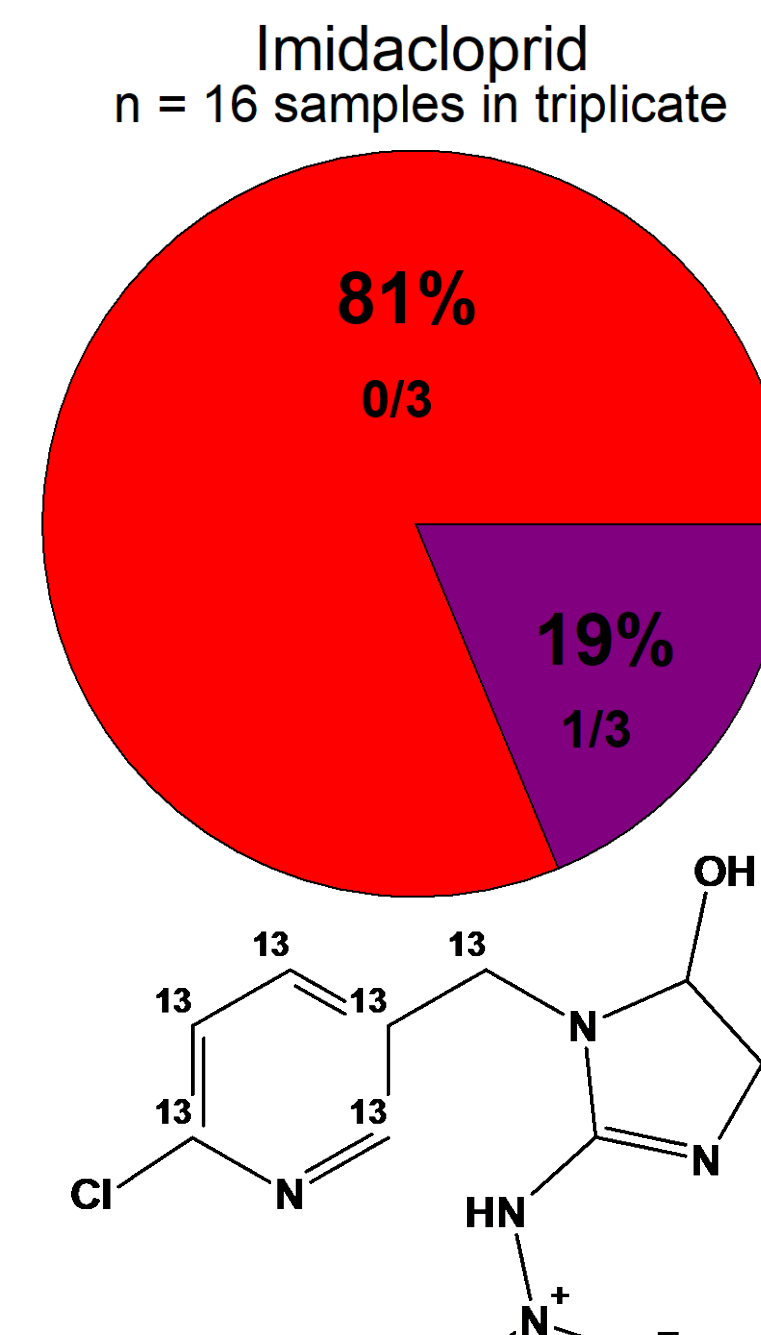
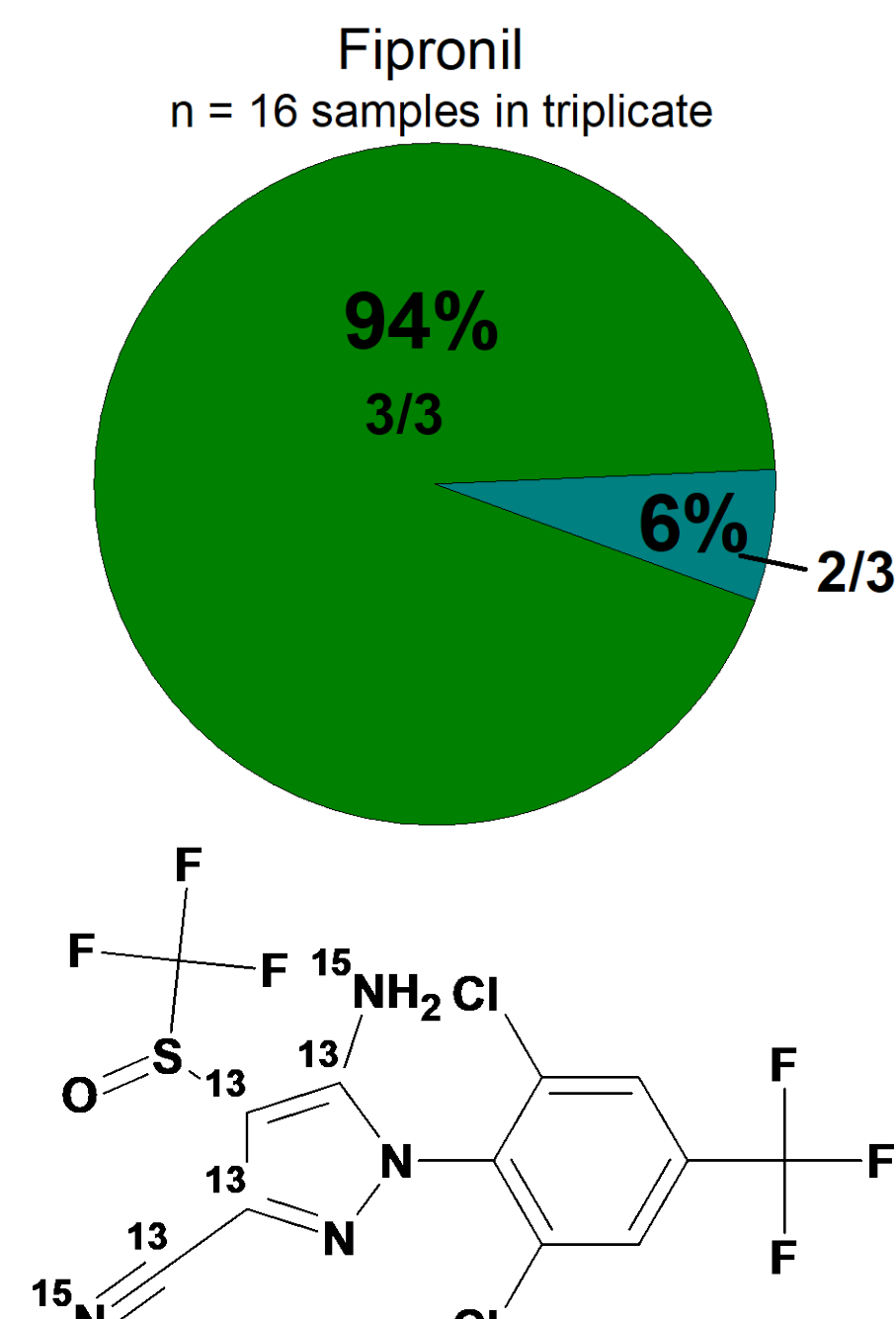
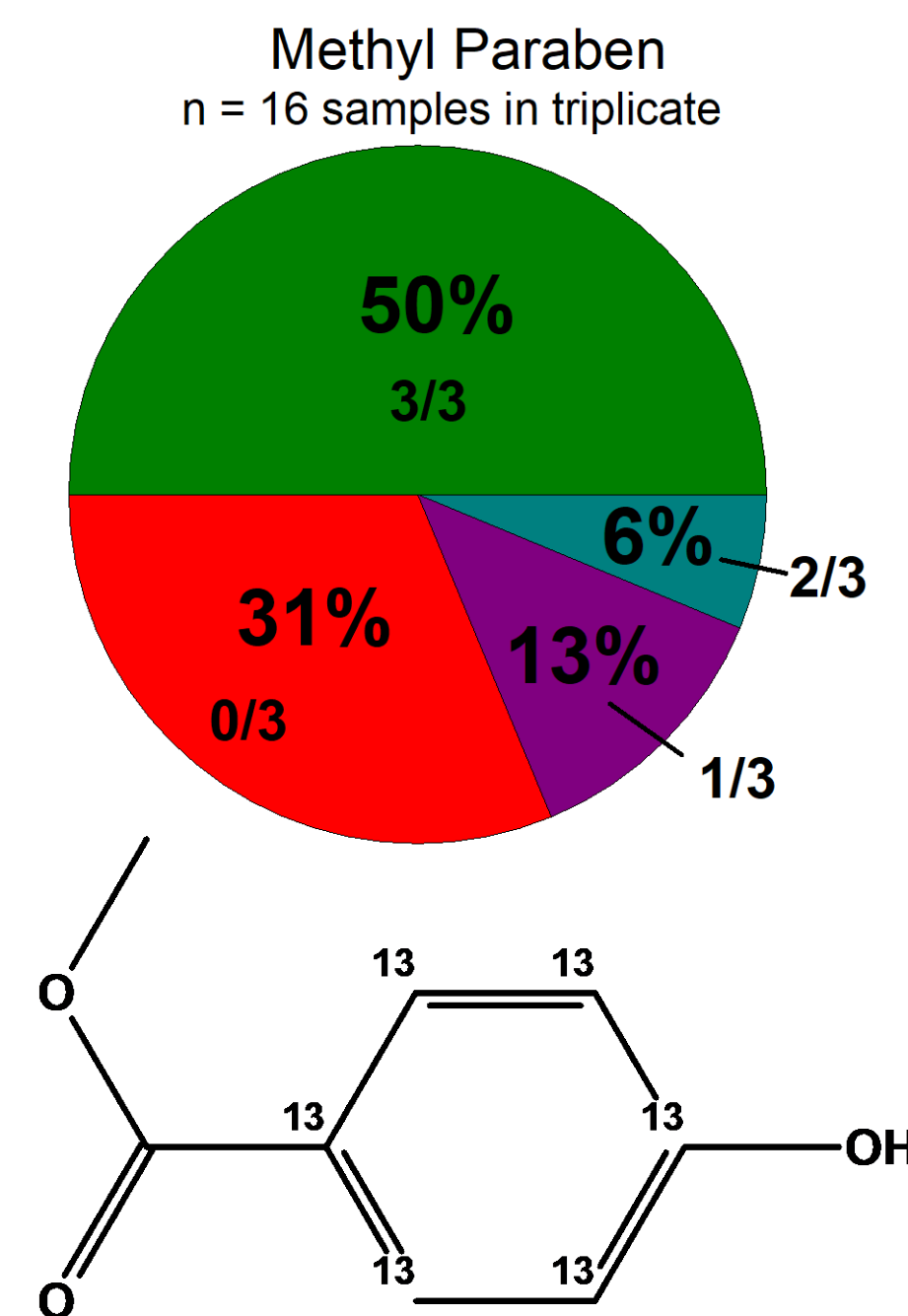
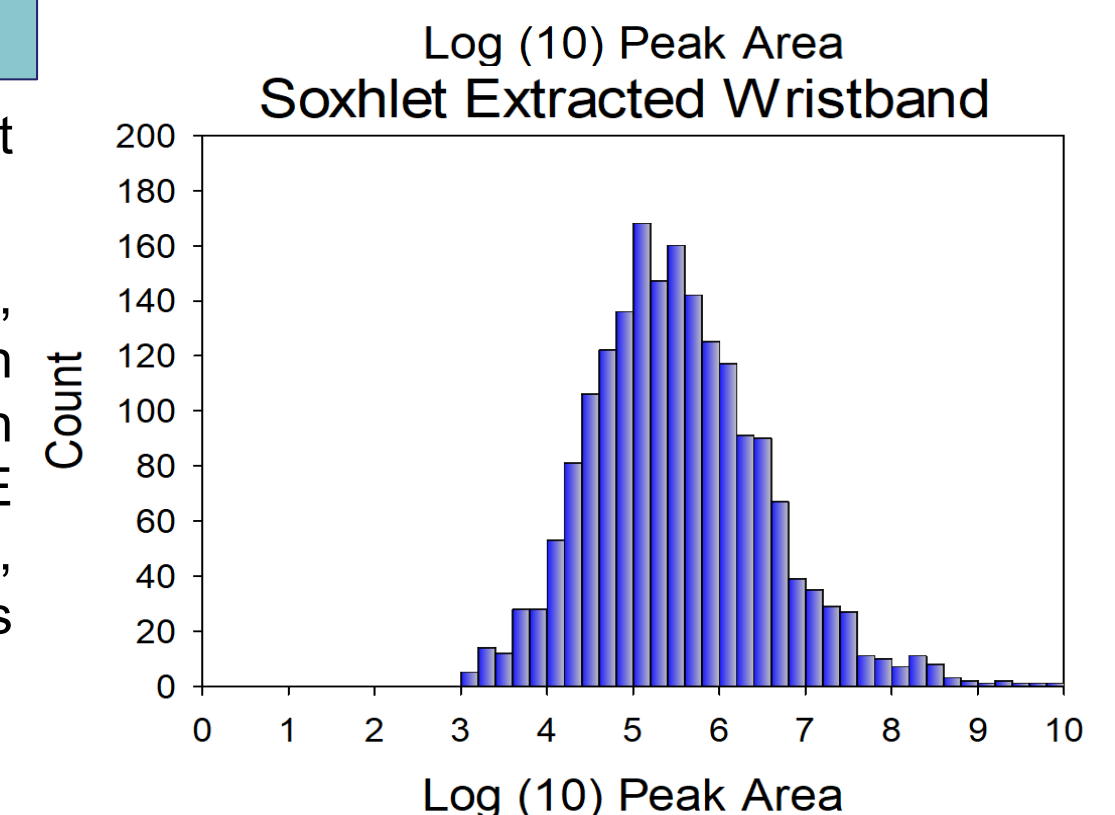
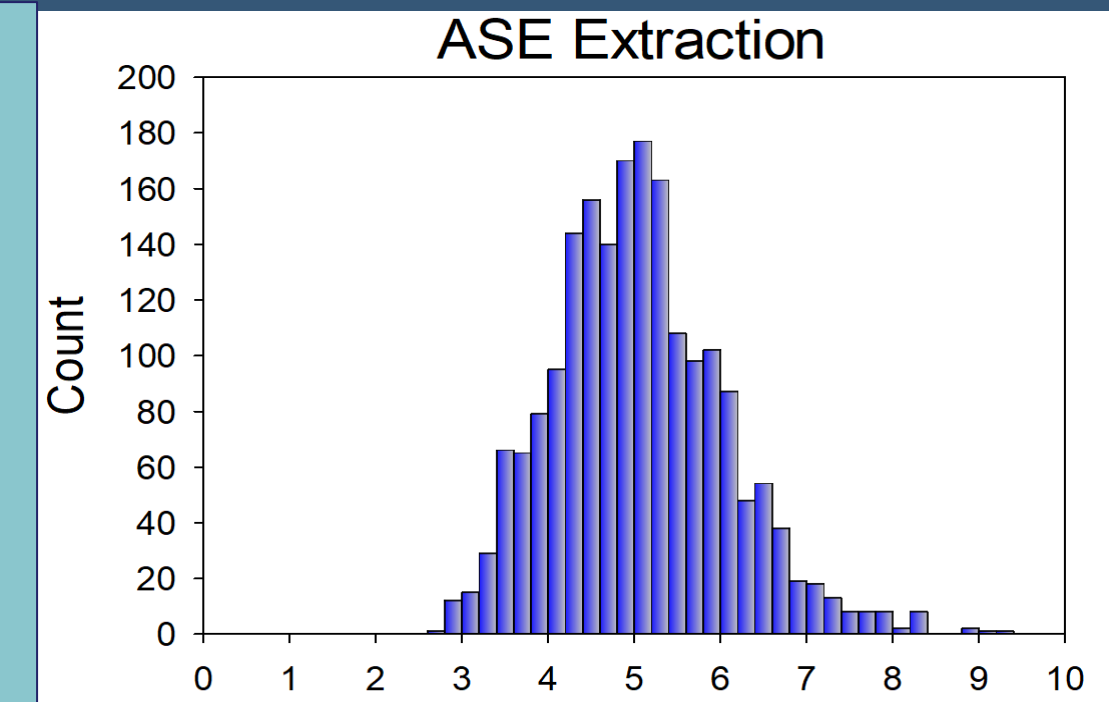
- Once cleaned, wristbands were stored in sealed plastic bags in a -20 °C freezer until extraction. Once extracted, samples were reduced to 1 mL rotary evaporation and additional evaporation under N<sub>2(g)</sub> and stored in GC sample vials @ -20 °C until analysis on a Thermo GC-Orbitrap Thermo Scientific (Waltham, MA) Trace 1310 gas chromatograph coupled to a Q-Exactive Orbitrap mass spectrometer
- As controls, uncleaned wristbands were extracted in each method to confirm the efficacy of cleaning the wristbands. Because extraction methods themselves may introduce contamination for NTA methods, extractions without the wristbands were conducted in tandem to wristband extractions. Isotopically labeled standards were spiked into the extraction vessel (e.g. Soxhlet body, ASE cells, etc.) directly before extraction. The standards, referred to as **Method Standards**, are used to evaluate recovery.
- Instrument performance was evaluated with a spike of labeled standards referred to as **Instrument Standards** into samples before analysis on a GC-Orbitrap. An additional standard mix made in ethyl acetate was made to confirm retention time.
- Additional instrument performance was evaluated by analysis of Soxhlet extracts of uncleaned wristbands donated by EPA staff. These bands were used as a worst-case-scenario for the instrument and were run in triplicate to determine QA/QC requirements.
- Results were evaluated for peak number, area, retention time, and mass accuracy using Thermo Xcalibur 4.4 and Compound Discover 3.3 software.

## Results

Number of GC peaks was used to determine the number of analytes present in the sample. While further research is needed to determine which oven cleaning methods are preferred, it is clear comparing the number of peaks in uncleaned vs. cleaned wristbands, that cleaning the bands has benefit. Vacuum oven was used for further experiments



Comparing number of peaks between extraction methods, more peaks is deemed to indicate a preferable extraction. These preliminary results indicate that Soxhlet extraction and ASE outperform both Orbital Shaker and Sonication. However, more experiments are needed to statistically differentiate.



The pie charts below indicate how often an Instrument standard was detected in a triplicate run of uncleaned silicone wristbands, donated by EPA staff. These wristbands are used only to determine QA/QC protocols, as these wristbands are considered a “worst-case scenario” for field samples. Above, the pie charts indicate how many times each Instrument standard was found in each sample throughout the analysis. Curiously, all three standards were not detected 100% of the time. Fipronil was the most consistent, followed by Methyl Paraben, and Imidacloprid, which was rarely identified.

|                | Retention Time (min) | Average Peak Height                         | n of detections in sample replicates | Mass Error (ppm) Samples | n of detections in standard mix | Mass Error (ppm) Samples and Standards |
|----------------|----------------------|---|--------------------------------------|--------------------------|---------------------------------|--|
| Methyl Paraben | 13.96 ± 0.05         | 6.66×10 <sup>8</sup> ± 1.94×10 <sup>9</sup> | 28                                   | 0.27                     | 7                               | 2.92                                   |
| Fipronil       | 20.05 ± 0.04         | 3.61×10 <sup>8</sup> ± 2.78×10 <sup>8</sup> | 47                                   | 317*                     | 7                               | 7.28                                   |
| Imidacloprid   | 21.00 ± 0.01         | 5.18×10 <sup>6</sup> ± 8.64×10 <sup>6</sup> | 3                                    | 414**                    | 5                               | 2.44                                   |

- The area of Method standards proved to be consistent across all extraction methods

- To decide between Soxhlet and ASE extracted wristbands, peak area of all detected analytes is compared in representative histograms. The number of peaks in both extraction methods are nearly identical, however ASE extracts produce lower peak area than Soxhlet extraction, favoring this method. Specifically, Soxhlet extractions maximize at areas 10<sup>5-6</sup> while ASE peak area is at 10<sup>4-5</sup>.

## Conclusions and Future Work

- Cleaning methods have some effect on silicone wristbands, though to a lesser extent than anticipated. Additional testing will be needed to indicate which cleaning method is best for NTA.
- ASE and Soxhlet perform well for extraction methods, and are hard to distinguish between. More data is needed to conclusively determine a preference, but for the sake of future work, Soxhlet will be used.
- When using standards with a variety of chemical properties (masses, boiling points, etc.), it is important to note that not all standards were detected consistently even in the same sample (triplicate runs). Peak intensity of Instrument standards was extremely variable and needs further investigation. Though all standards that were detected showed excellent consistency in retention time, mass accuracy is consistent for 3/3 instrument standards when detected. The same cannot be said for samples where only 1/3 instrument standards remains consistent.
  - \*Currently we are unsure why Fipronil has a discrepancy in extracted samples versus a standard mix of the instrument standards.
  - \*\*We are concerned that the identity of imidacloprid in the three samples is inaccurate and possibly causing the high mass error.

To continue this work, silicone wristbands will be deployed in the field (n=5 participants) for preliminary understandings of method reproducibility and to find potential foci for larger scale studies utilizing the vacuum oven cleaning method and Soxhlet extraction.

## References

Thanks to the NSF: INTERN program for funding this research. (1) Anderson, K. A., et al. (2017). *J Expo Sci Environ Epidemiol* **27**(6): 551-559. (2) Donald, C. E., et al. (2016). *R Soc Open Sci* **3**(8): 160433. (3) Kile, M. L., et al. (2016). *Environ Res* **147**: 365-372.