

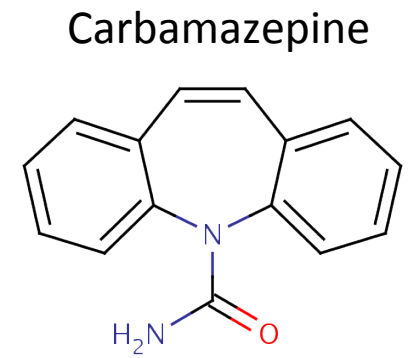
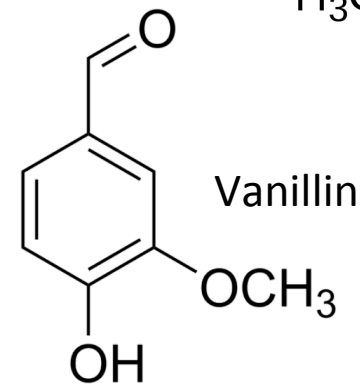
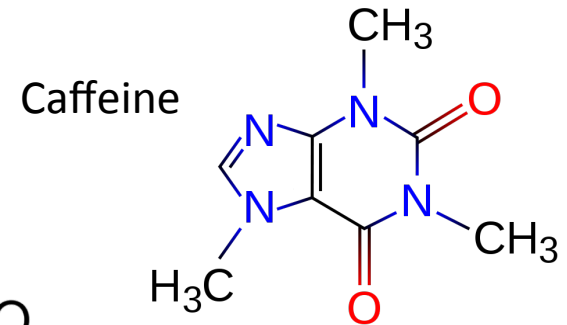
# Determination of Water Sample Stability as a Function of Storage Conditions

Derek Overman



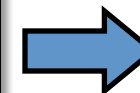
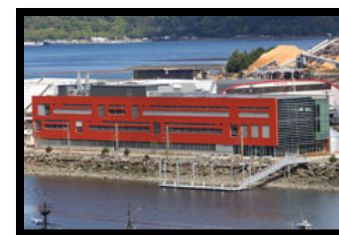
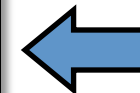
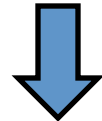
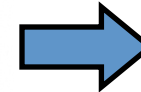
# Sound Citizen Program Logistics

- Citizen science program
  - Community based
  - Sample kits may be sent to unknown individuals
  - We anticipate and rely on individual's proper sampling techniques and returning the sample promptly
- Students, organizations, other groups, and individuals collect water samples
- Samples are analyzed for specific, human influenced compounds
- Sample data is returned to sample collectors



# Sampling Logistics

- Sampling kits are requested via SoundCitizen.org
- Sample kits are sent with collection bottle, gloves, instructions, sample data sheet, and a pre-paid postage box
- Samplers collect samples and store samples in corresponding boxes
- Samples are shipped to Sound Citizen at CUW. Samples are either refrigerated or filtered immediately
- Collected samples may spend an extended amount of time exposed to unknown conditions

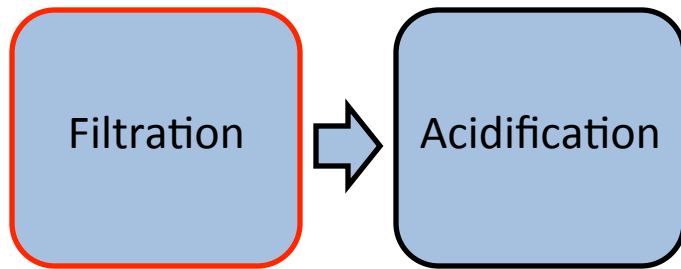


# Shipping Times

- Sample return time is thought to be important to sample quality
- Sample exposure to conditions is unknown
  - Individuals collecting samples are sometimes anonymous, lack sampling training
  - Shipping errors
- Upon arrival, samples are either immediately refrigerated or filtered, acidified, and frozen
- Sound Citizen at UW Seattle informed samplers that their samples would be discarded if not received within 7 days of sampling

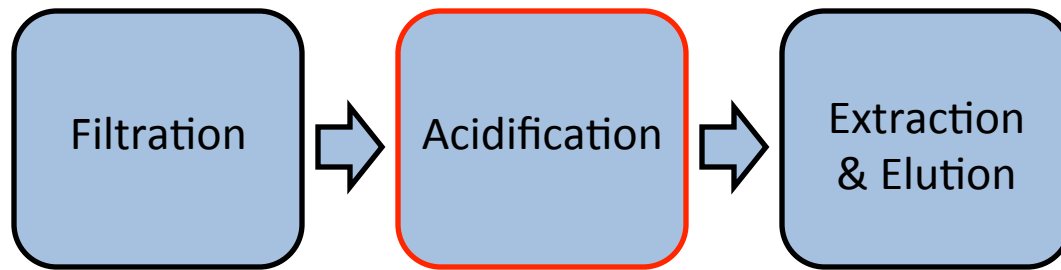
# Analytical Method: Overview



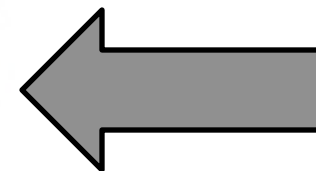
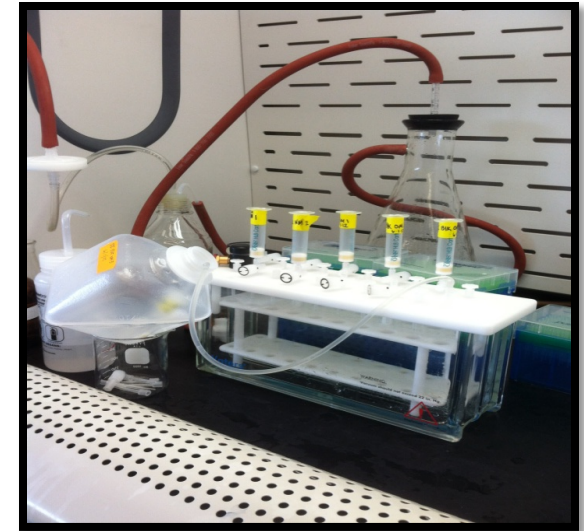


- Filtration
  - Samples are filtered through 0.7  $\mu\text{m}$  and 0.45  $\mu\text{m}$  filters.
  - Prevents clogging of compound collecting cartridges.
- Acidification
  - Sample volume is calculated and is acidified to pH 2 with concentrated HCl.
  - Acidification allows for prolonged storage while frozen.





- Solid Phase Extraction / Elution
  - Compounds collected on hydrophobic cartridge from water sample
  - Cartridges are eluted with 8 mL MeOH into test tubes



Hydrophobic cartridge



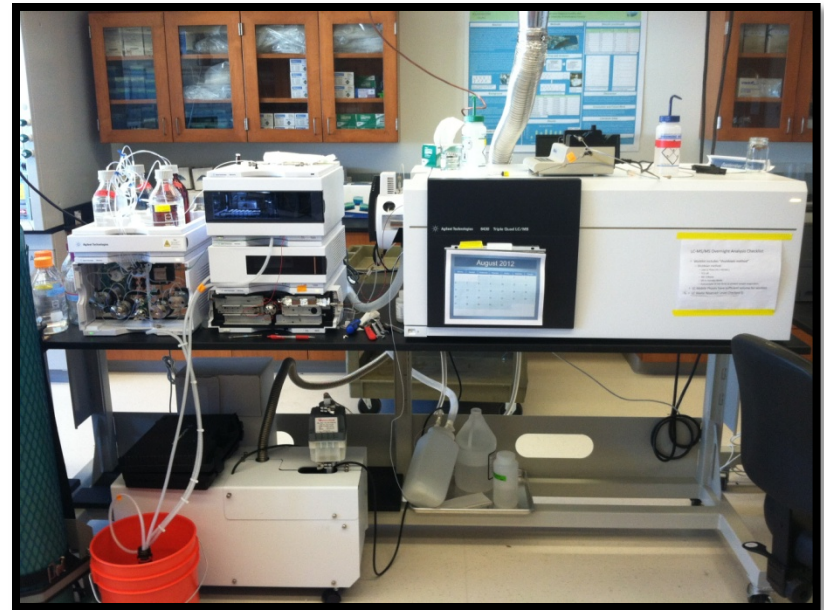
- Evaporation
  - Samples are evaporated to 1mL with nitrogen and 35 °C water bath
- Preparation
  - Samples are prepared in 1.5 mL amber vials and acidified with 250µL pH 2.8 acetic acid







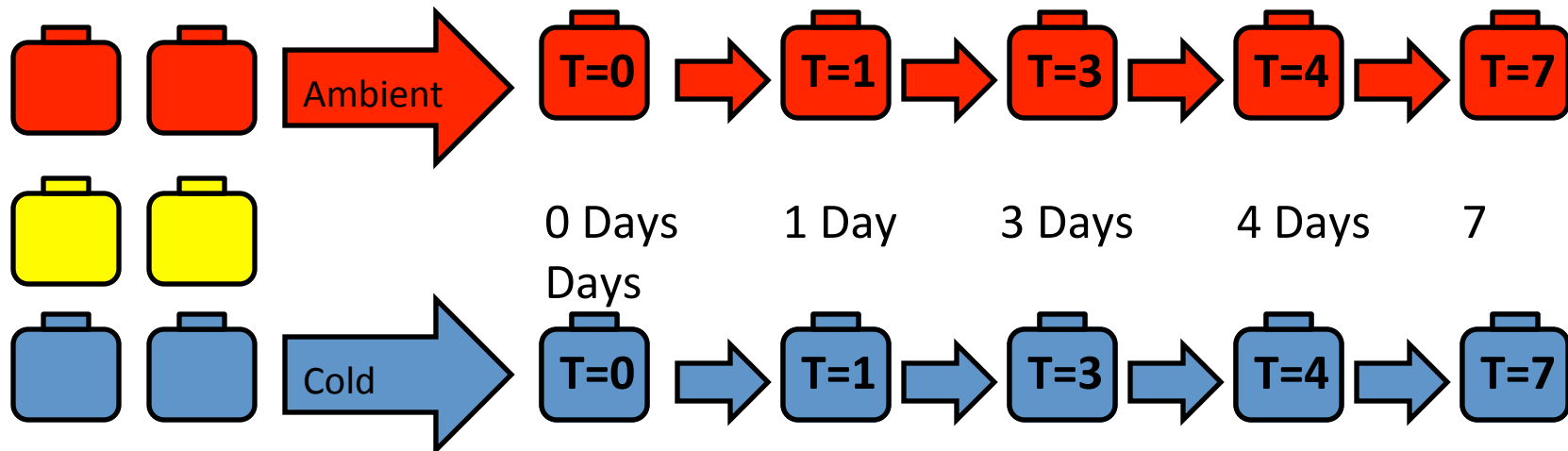
- Compound concentration is measured using Triple Quad High Performance Liquid Chromatography Mass Spectrometer.



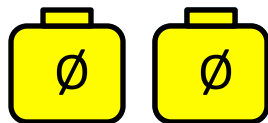
# Objective & Hypothesis

- Objective: To determine the relative stability of sample compounds in ambient and cold conditions.
- Hypothesis: Samples with prolonged exposure to ambient conditions will result in lower stability as compared to samples kept cold.
- Degradation: Samples may experience loss in compound recovery.
  - Chemical decay
  - Bacterial growth
  - Oxidation of compounds
  - Evaporation of compounds

# Experimental Design


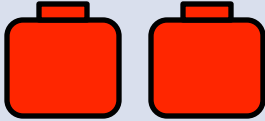







- 24 samples collected from CUW dock.
- 20 samples spiked with known amounts of compounds
- Spiked samples assigned known times of storage in days
- Samples divided equally into 2 groups of 10, ambient and cold.
  - Ambient stored in dark, room temperature cabinet
  - Cold samples stored in dark at 5° C.



- 4 sample blanks were not given compounds.

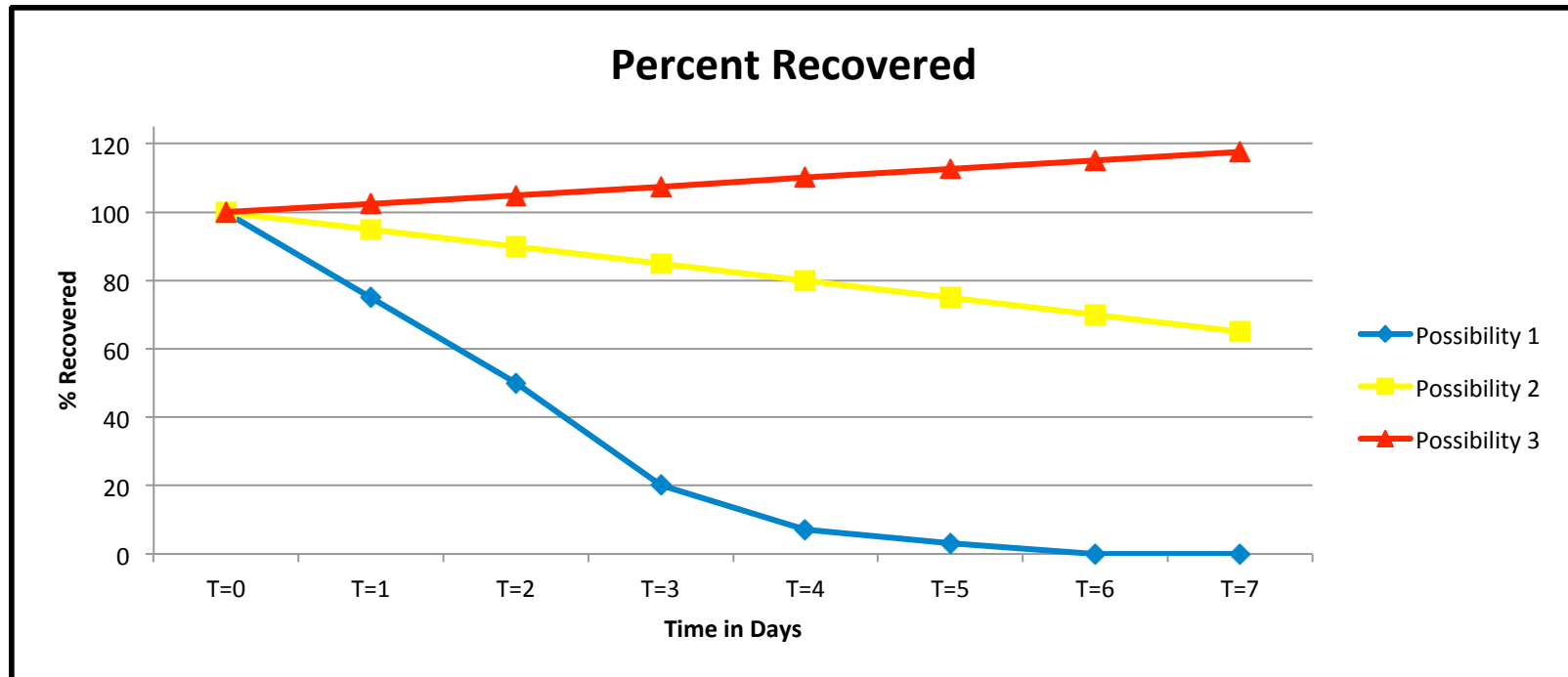
# Experimental Design

Time	Cold	Ambient
T=0		
T=1		
T=3		
T=4		
T=7		

- Individual time points (each day) received 4 samples total
  - 2 cold samples
  - 2 ambient samples
- Each time point was extracted with DI water blanks on each pre-determined day
- All samples were analyzed together after final time point (T=7) was extracted.

# Expectations

- Compounds may show different rates of compound stability.
- Samples may undergo rapid loss of compound recovery.
- Samples may show relatively stable recovery.
- Some concentrations may increase due to similarity of compounds.
  - Example: Salicylic acid & acetyl salicylic acid



# Results

- Operational Issues
  - All cold samples were filtered in about 15 minutes for all time points (T=0 through T=7) as well as time points T=0 and T=1 for the ambient temperature samples
    - Normal filtration takes about 15 minutes
  - At time point T=3, ambient sample filtration and extraction began to occur at a decreased rate
    - Each ambient T=3 and T=4 sample took about 2 hours to filter
  - By time point T=7, filtration and extraction times for ambient samples were significantly longer than cold samples.
    - Ambient T=7 samples took over 3 hours to filter and extraction did not complete for the entire sample

# Percent Recovery

- To determine stability, a percentage of recovery was calculated

$$\% \text{ Recovery} = \left( \frac{\text{Avg. Conc. @ T=1 Day}}{\text{Avg. Conc. @ T=0 Days}} \right) * 100$$

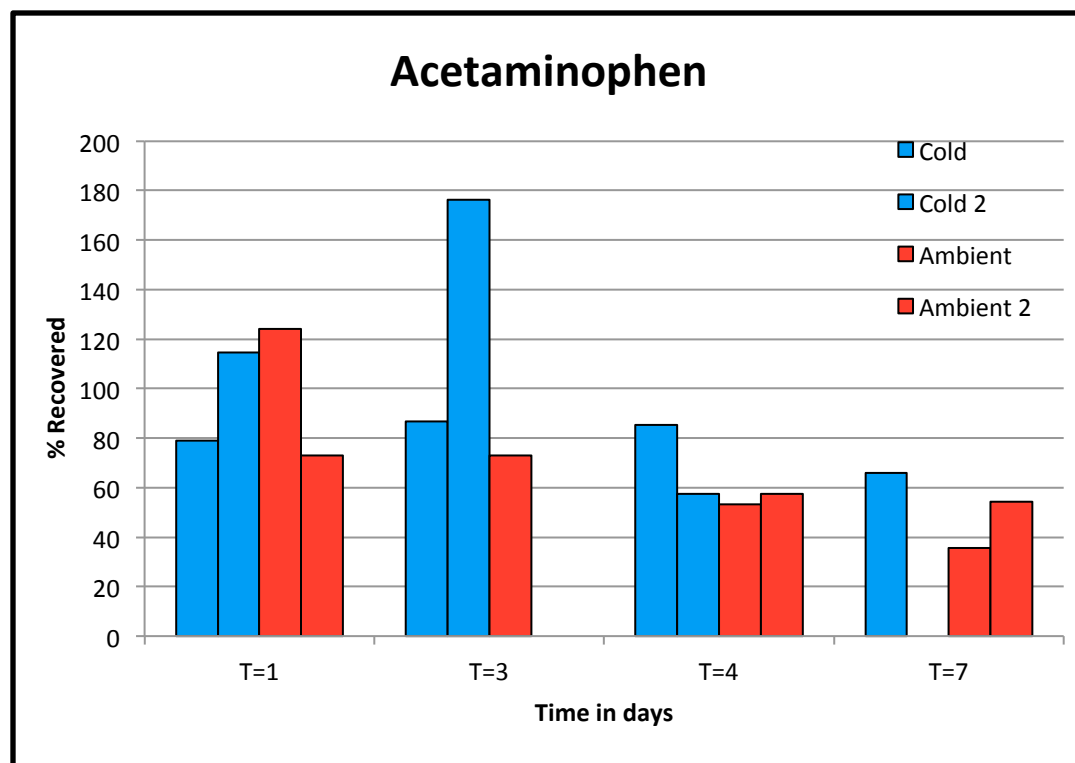
# Recovery

Storage Time	Average Caffeine Detection (ng/ml)		Caffeine % Recovery	
	Cold	Ambient	Cold	Ambient
<b>T=0</b>	0.3388		100	
<b>T=1</b>	0.2713	0.2795	80.1	82.5
<b>T=3</b>	0.3761	0.279	111	82.4
<b>T=4</b>	0.2556	0.2216	75.4	65.4
<b>T=7</b>	0.2327	0.2276	68.7	67.2

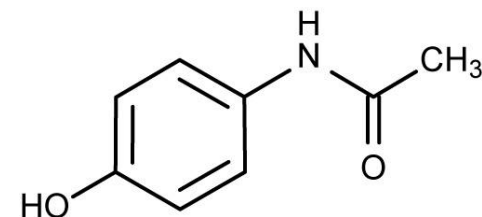


# Results

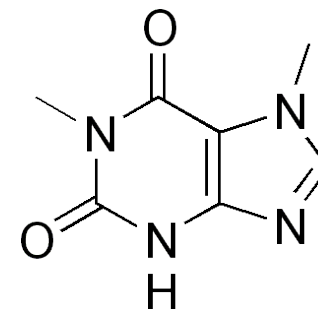
- Some compounds show gradual recovery decrease amongst both sample conditions.



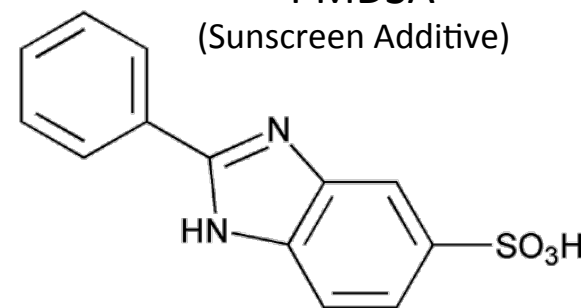
Acetaminophen



Paraxanthine

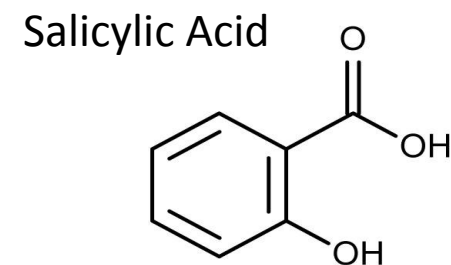
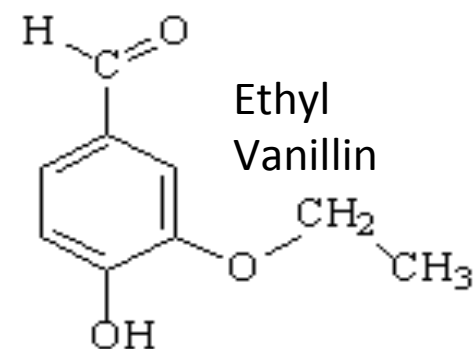
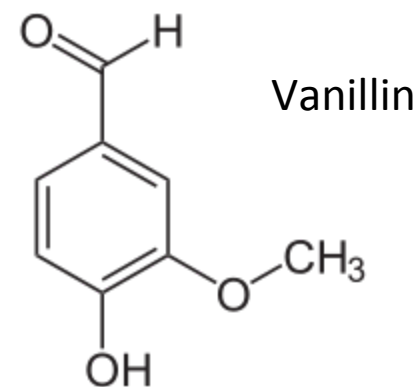
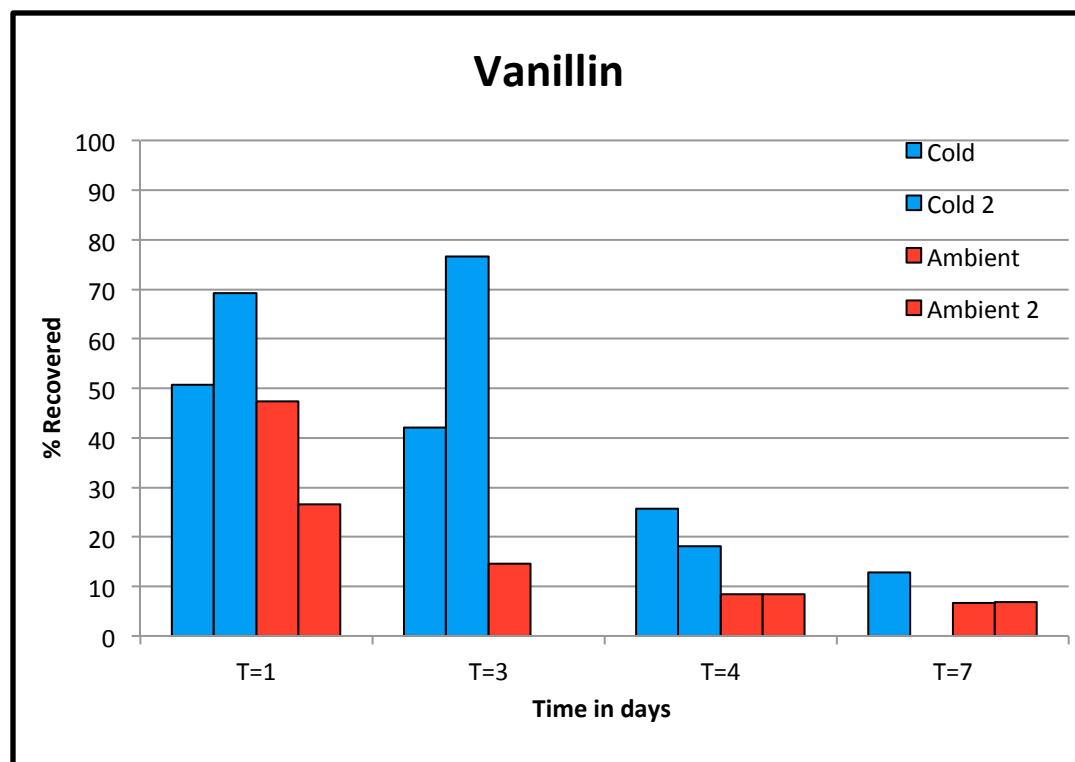


PMDSA  
(Sunscreen Additive)



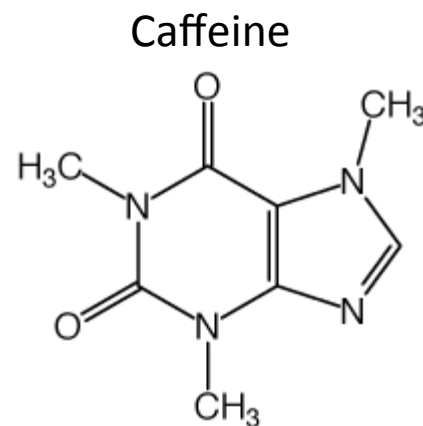
# Results

- Vanillin, ethyl vanillin and salicylic acid showed significant decrease in recovery for both conditions, but was more dramatic for ambient conditions.

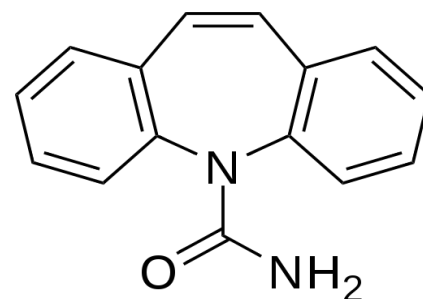


# Results

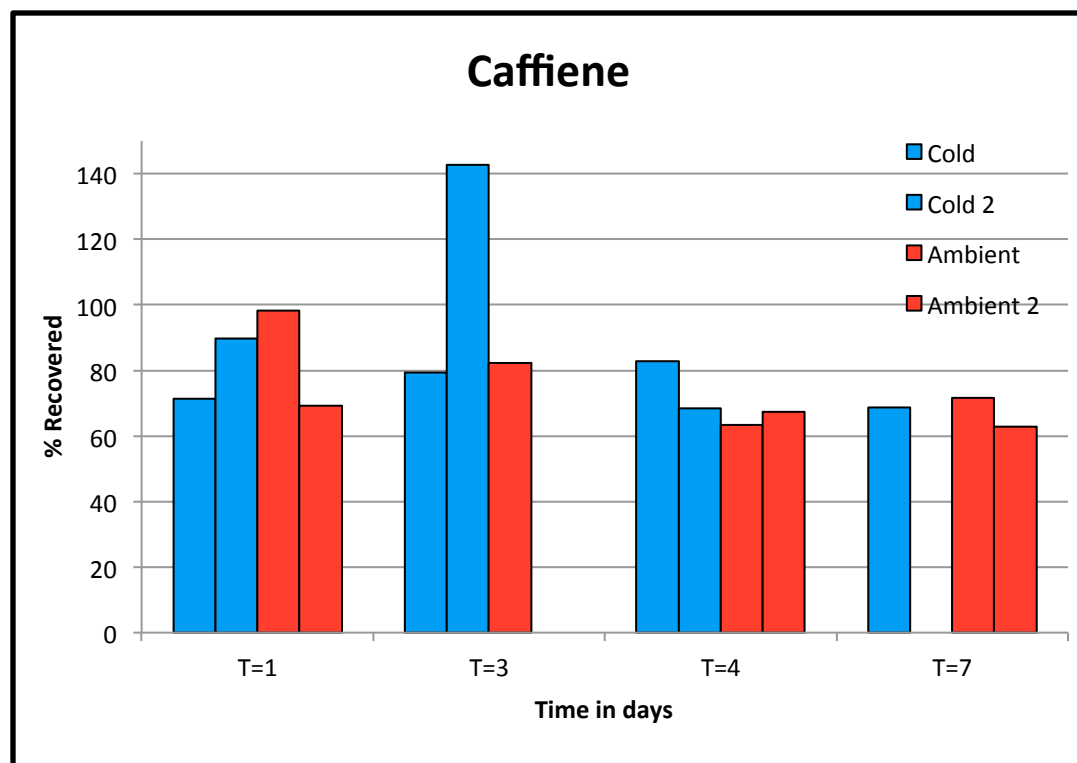
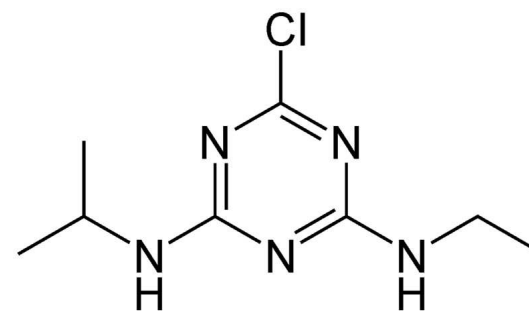
- Caffeine, carbamazepine, and atrazine all remained relatively stable in either condition.



Carbamazepine



Atrazine



# Discussion

- Possible reasons for varied results amongst compounds:
  - Similarity of compound structure
  - Function of compounds
  - Similarity of interactions of compounds with water
  - Octanol-Water Partition Coefficient
  - Boiling Point
- Operational Issues
  - May be related to bacterial growth or precipitation of some larger solids, although samples were not visibly different from samples that ran normally.

## Ambient Condition Samples

Compound	> 40% Recovered		> 60% Recovered	
	T=4	T=7	T=4	T=7
	Atrazine	√	√	√
Caffeine	√	√	√	√
Carbamazepine	√	√	√	√
Acetaminophen	√	√		
Paraxanthine	√	√		
Propylparaben	√	√		
PMDSA	√	√		
Ibuprofen	√	√	√	
Vanillin				
Ethyl Vanillin				
Salicylic Acid				

# Conclusion

- Stability for ethyl vanillin, vanillin, and salicylic acid was low
  - Over 60% of these compounds were lost in 1 week
- Stability for acetaminophen, paraxanthine, propylparaben, PMDSA, ibuprofen, was relatively good
  - Less than 60% were lost after a week of storage in ambient conditions
- Stability for atrazine, carbamazepine, and caffeine was good
  - Less than 40% were lost after 1 week of storage in ambient conditions

# Conclusion

- Samples exposed to 1 week of unknown temperature conditions can be analyzed with minor operational issues and recovery of some of our detectable compounds
  - Samplers can still receive data, even with a week delay in sample return
- Data for our more stable compounds can be given and used with more confidence

# Next Steps

- Multi-week stability experiment
  - Useful for non-domestic samples or extended sample transport delay
- Acidity experiment
  - pH 2 & frozen state stability
- Photo experiment
  - Compound stability in light



# Special Thanks To

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**W**

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