Aerobic Decomposition - Alternative Method for Managing Large-Scale Animal Fatalities

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Project Description

Purpose:

The general goal for this research is the creation of an end product that will provide a scientifically proven method for the appropriate disposal of deceased animals in high-magnitude mass fatalities that is acceptable and cost efficient.

Relation to Homeland Security:

The disposal of mass fatalities of large animals must be handled in a manner to prevent the spread of zoonotic disease and protect the environment and water supplies.

Capability Requirement Gap

- Tennessee state regulations require proper disposal of animal carcasses.
- Composting, burial, and incineration are methods used to dispose of large animal carcasses. This work is designed to elucidate the development and disposition of decomposition products and determine the safest manner of disposal.
- The data derived from this study will allow for the safer handling of carcasses by knowing what is present and also for possibly being able to decontaminate carcasses by chlorine dioxide and bacterial degradation of pentobarbital.
- This project investigated different materials for decomposition, identification of bacteria capable of degrading pentobarbital and the decontamination of carcasses by chlorine dioxide.
- Cost: $160,708
- Benefit: the prevention of an epidemic due to zoonotic disease from contaminated carcasses.

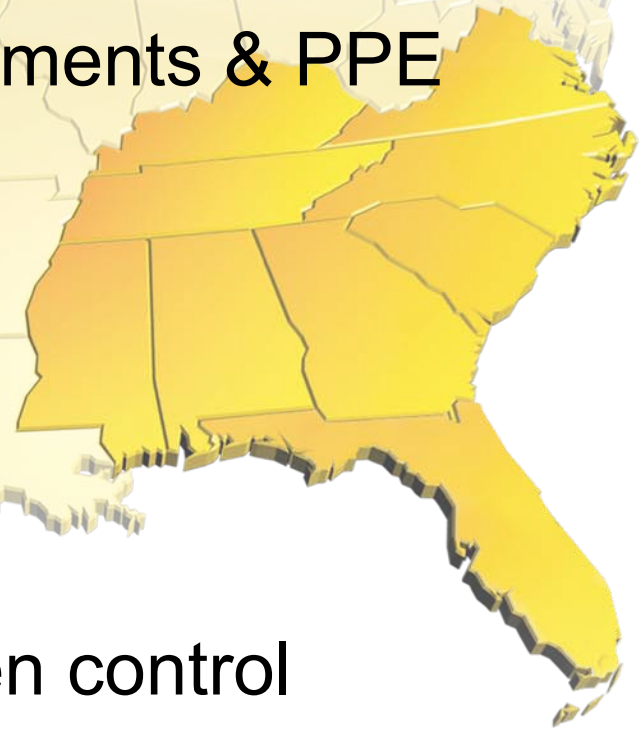
Chlorine Dioxide

Anthony Newsome PhD

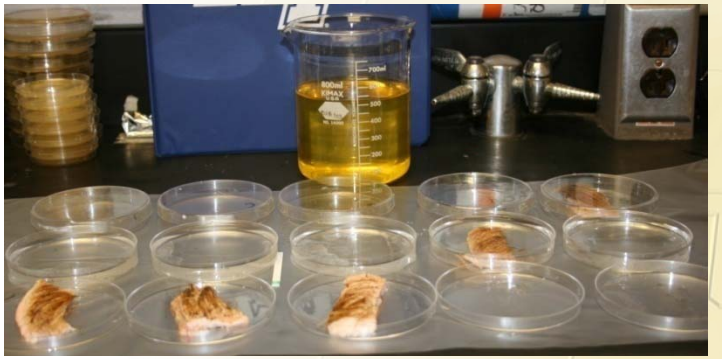
- Discovered in 1814
- Commercial use for water treatment and bleaching (1940s)
- EPA registered:
  - liquid form as disinfectant (1967)
  - gas form as sterilant (1988)
  - approved by EPA for Antrhax decontamination
    - -Used for Anthrax decontamination of federal office buildings in 2001
- Instability of gas prohibits transport - must be prepared onsite
- New generation technology provides easy on-site chlorine dioxide generation (such as ICA/TriNova system)

Chlorine Dioxide Research at MTSU

- Sterilization of medical instruments & PPE
- Football equipment
- Building materials
- Water treatment
- Foodborne Illness – Pathogen control



Testing Setup



Dip Tests

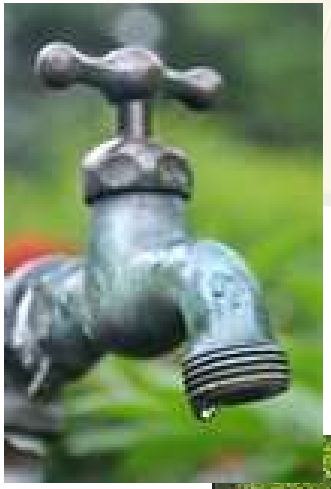
Spray Tests

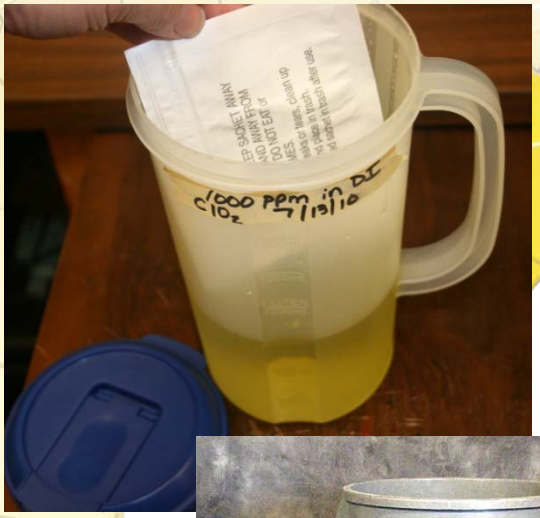
Gas Tests

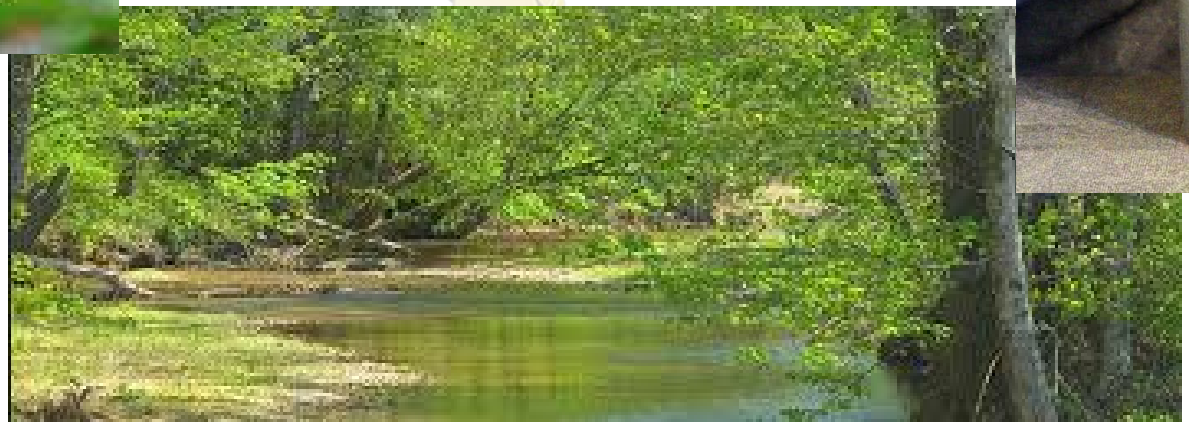
Solution Preparation

Easy to generate on-site

Can use a variety of water sources and containers





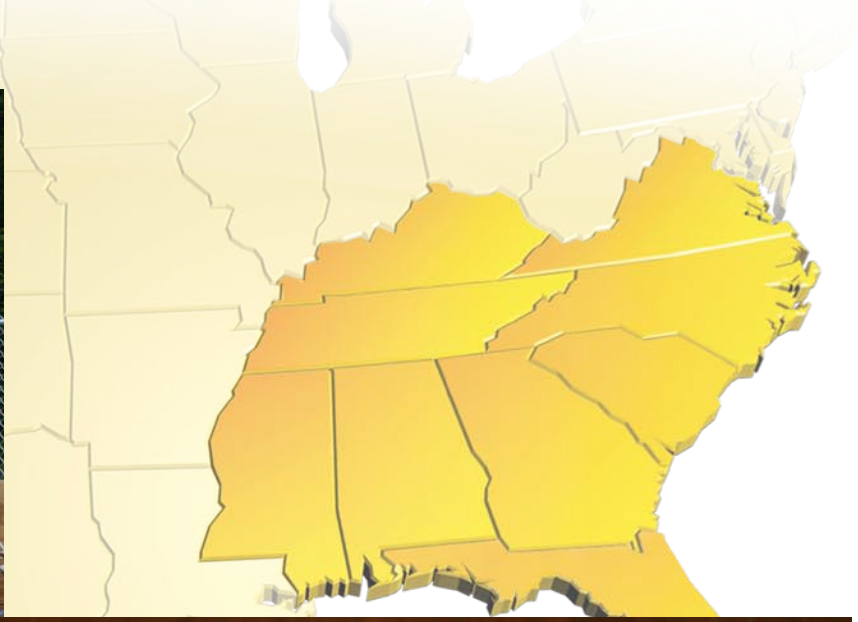


Sampling Methods

Untreated Samples

TSA Rodac Plates Used to Sample Pig Skin
(Plates incubated 24hrs at 37C + 24 hrs at room temperature)

Sampling Methods

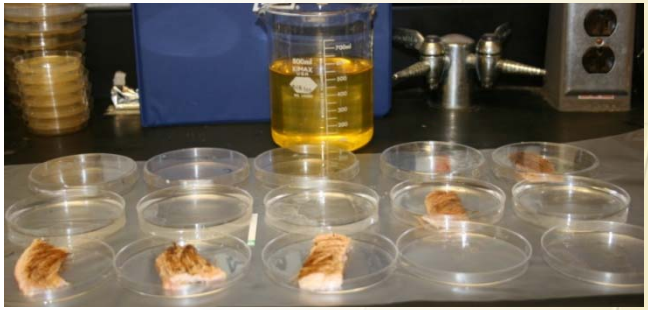


Skin Samples

- *Bacillus atrophaeus* (from Apex Laboratories) added to surface of skin samples as surrogate for *Bacillus anthracis*

- Serial 10-fold dilutions of homogenized samples used to estimate existing CFUs before treatment

Dip Test Results



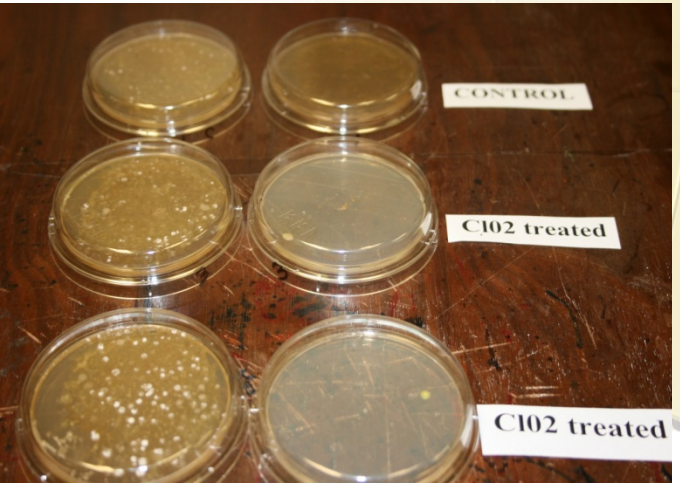
- Marked reduction in growth
- Treatment Times
- Concentrations

Spray Results

- Marked reduction in growth
- Solution may not penetrate into skin layers and hair follicles well without agitation

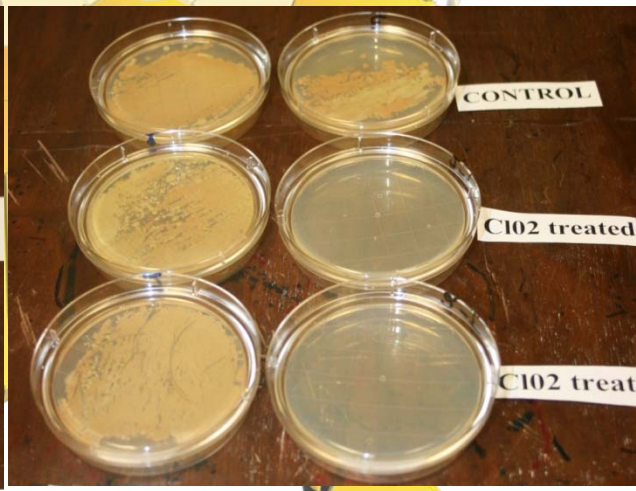
Spray Tests with 500 ppm solution

Gas Results at Various Temperatures



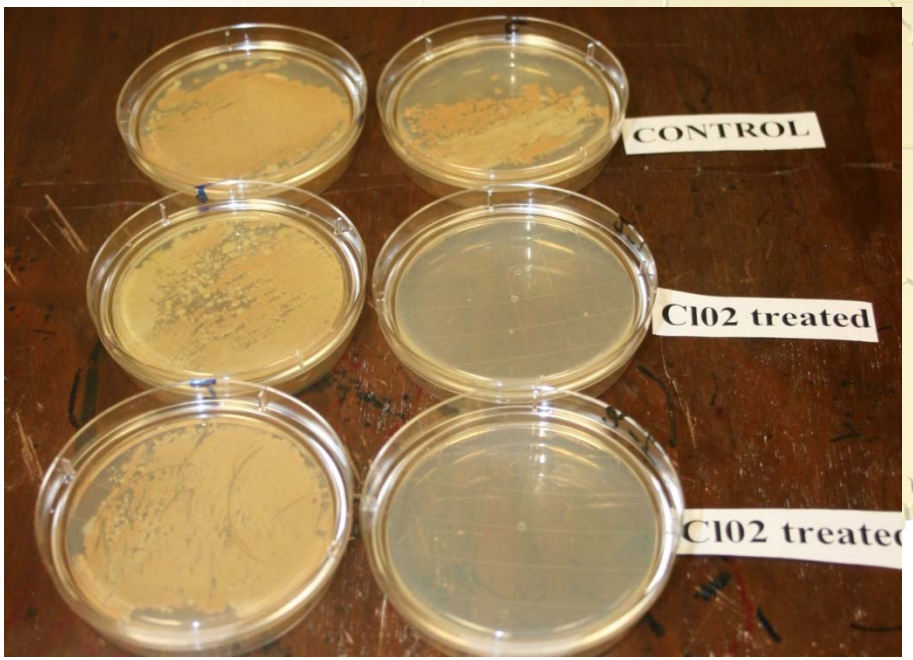
Gas Treatment at 4C

Gas Treatment at 22C

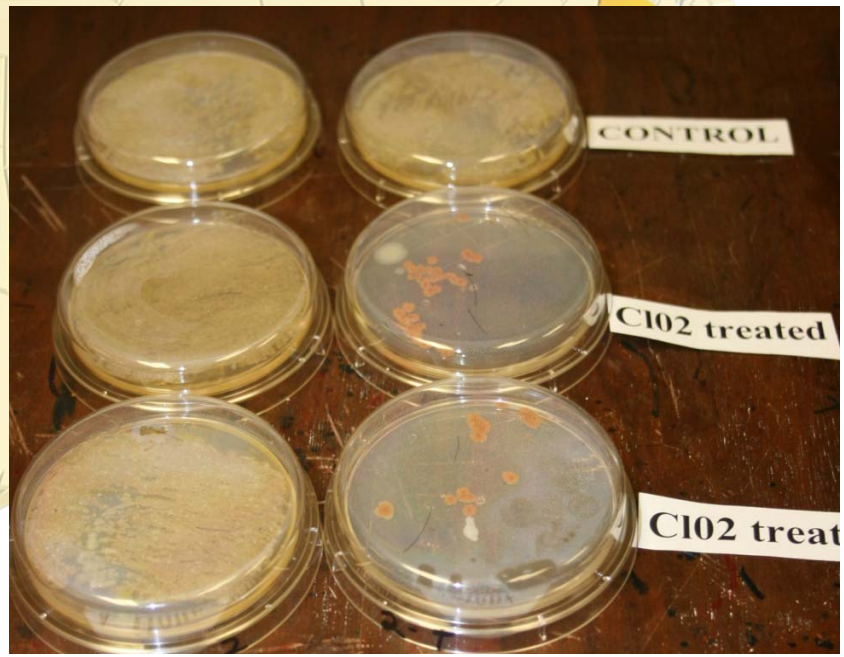


Gas Treatment at 32C

Gas Results – 32 C Tests



8 Hour Gas Treatment at 32C



1 Hour Gas Treatment at 32C

Optimization of treatment protocols
(length of treatment and concentrations needed at different temperatures)

Lessons

Skin is a unique surface to decontaminate.

Solutions are easily generated using a variety of water sources. Dips and sprays are effective in reducing growth (including spores) but unlikely to generate a total kill due to difficulty in penetrating to skin surfaces where pathogens can hide.

Spray of solution is likely more appropriate for large scale treatment than dipping method. Sprayed solution provides significant reduction in growth which may improve with optimal concentrations, repeated applications or treatment times. However, at this point we have not been able to generate a total bacterial kill with this method.

Solution may be an appropriate alternative when gas is not an option, such as decontamination of workers involved in handling infective carcasses or decontaminating surfaces than cannot be enclosed.

Gas form is easy to use and an extremely efficient form of decontamination which gives total kill with appropriate time, temperature and concentration protocols.

- Effective against range of pathogenic bacteria, including spores
- Variable reaction rates and gas behavior at various temperatures
- Gas form is preferred treatment method when enclosure can be achieved

Next Steps

- **Optimize Treatment Protocols**
  - **Spray & Gas**
  - **Environmental variables (temperature, sunlight, etc.)**
- **Larger scale test**
  - **Application to bags, tents, container cars, and other large enclosures**
  - **Identify mass and volume variables (carcass size & quantity)**
- **Effects of treatment on decomposition**
- **Applications to agriculture**
  - **Irrigation with manure slurry, livestock runoff, food processing, etc.**

Gaseous Compounds Produced During Decomposition

Ngee Sing Chong PhD

- Four 200 lb pigs were used for the decomposition study. Each were buried in different media: sand, soil, and sawdust. One pig was not buried and used as a control. They were buried on May 13, 2010 and gas samples have been collected for analysis for ten weeks.
- Each pig was sampled using gas canisters, evacuated bottles, and Tedlar® bags. Samples were taken biweekly for the first four weeks and weekly thereafter.
- Each sample was analyzed by Agilent Technologies' 6890 GC coupled to a 5973 MSD via a Nutech autosampler and preconcentrator system.

Day 5 Control Pig

Day 60 Control Pig

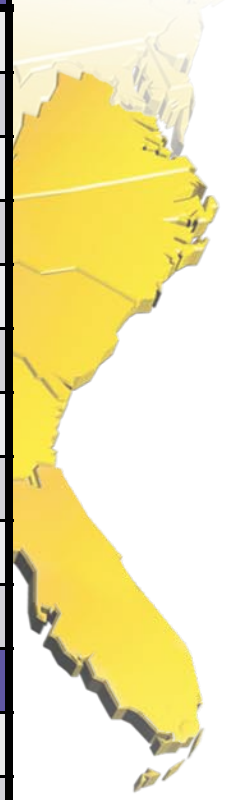
Gas Canisters

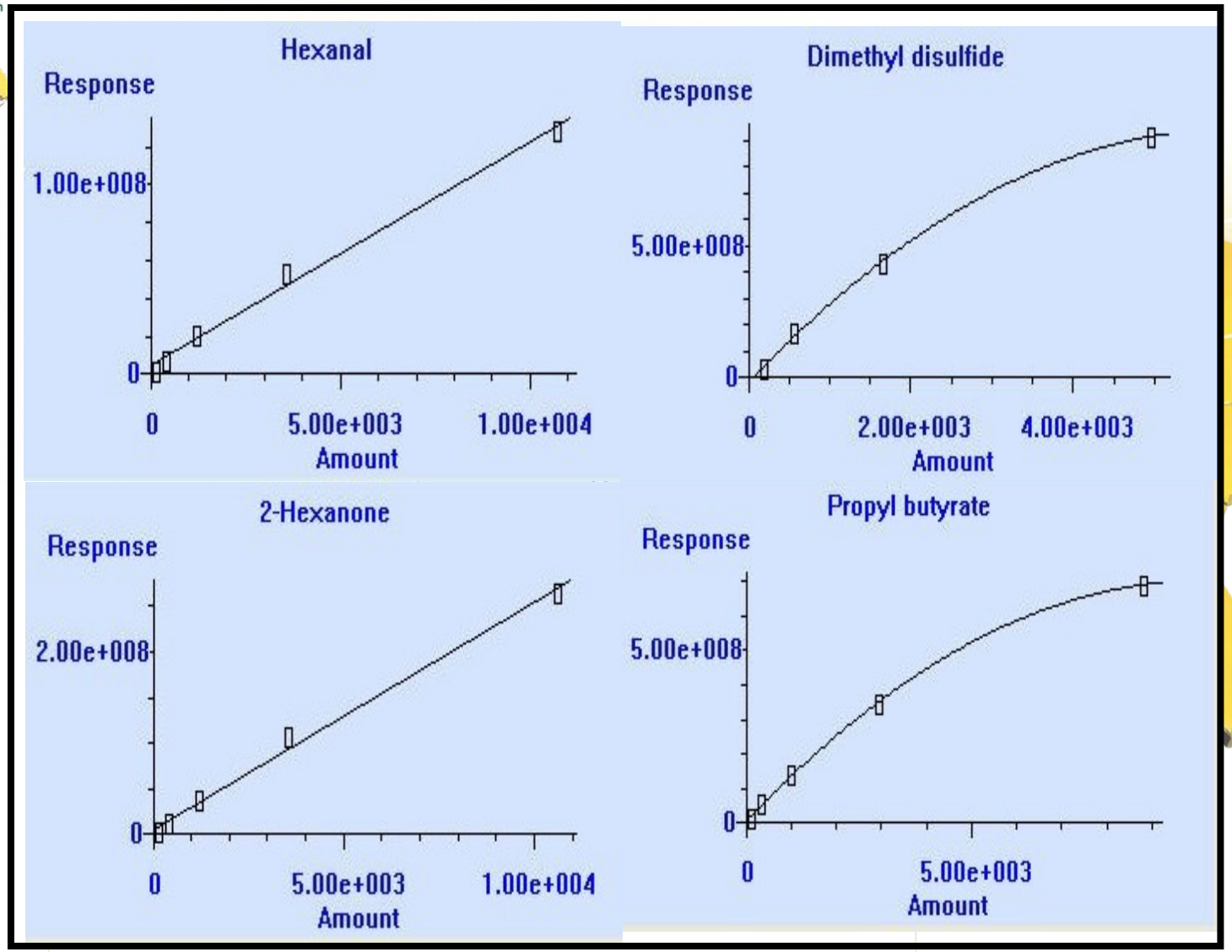
Tedlar ® Bags

Bottle Vacs

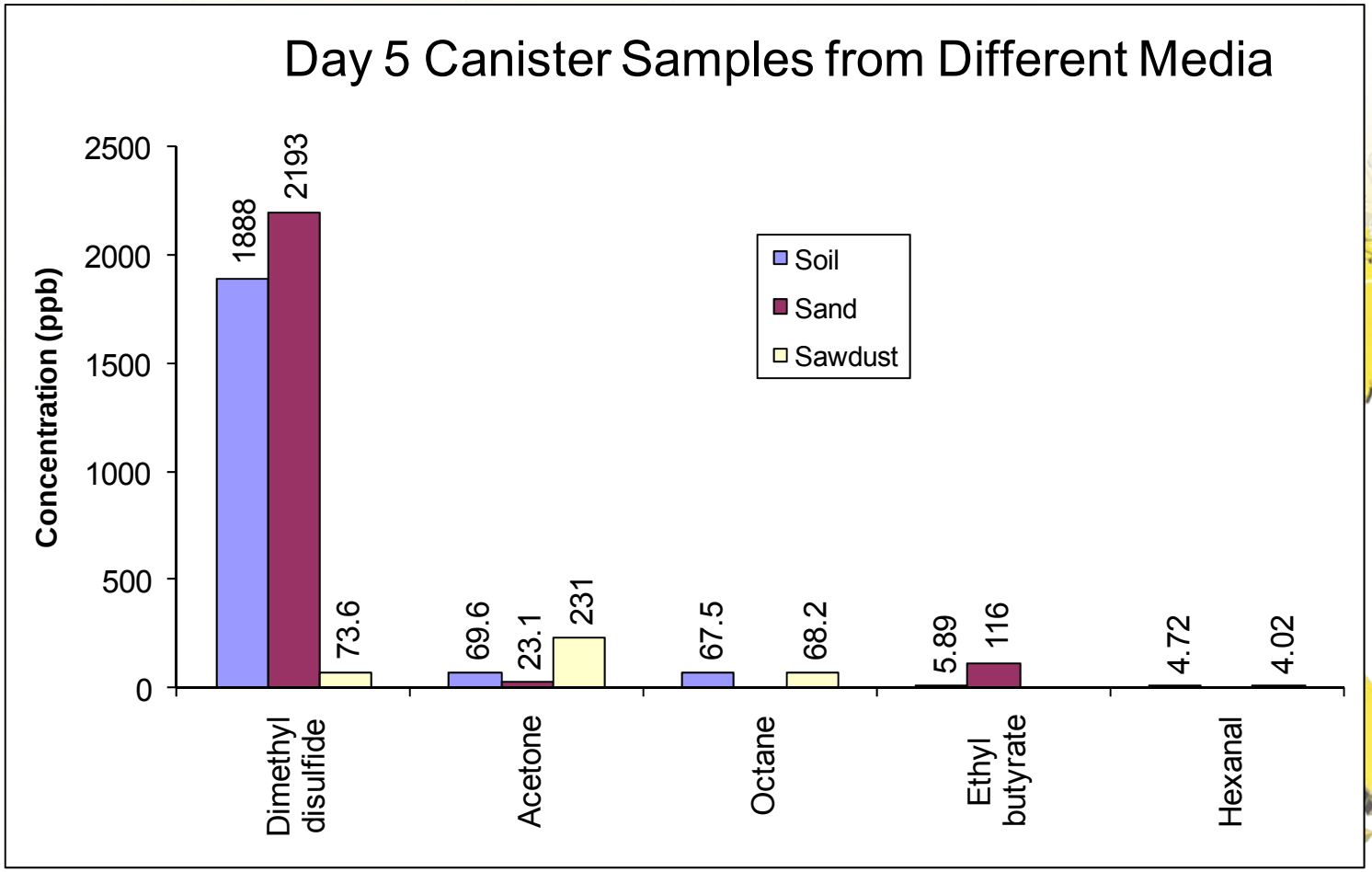
Sampling Methods

GC Method Conditions	
Initial Oven Temp and Time	32°C for 8 min
Ramp 1 Rate	5°C/min to 150°C, 1 min hold
Ramp 2 Rate	20°C/min to 300°C, 1 min hold
Column Type	Restek MTX-1
Column Length	60.0 m
Column Diameter	250 µm
Column Film Thickness	0.50 µm
Helium Carrier Flow	1.5 mL/min
Column Pressure	22.66 psi
Linear Flow Velocity	32 cm/sec
Preconcentrator Conditions	
Glass Bead Trap	Cools to -150°C Desorbs at 80°C
Tenax® Multimedia Trap	Cools to -20°C Desorbs at 200°C
Cryofocuser	Cools to -150°C Desorbs at 200°C



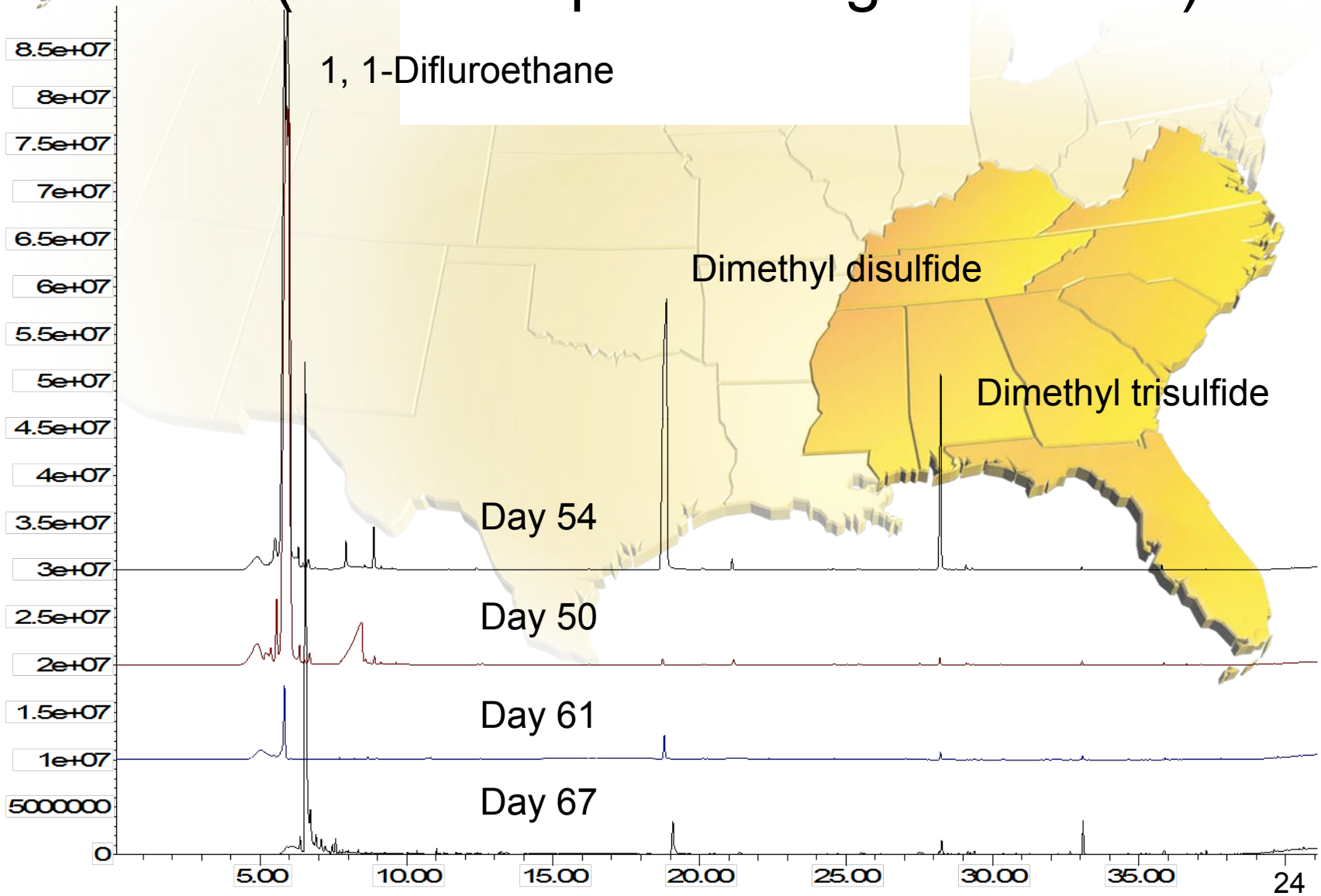


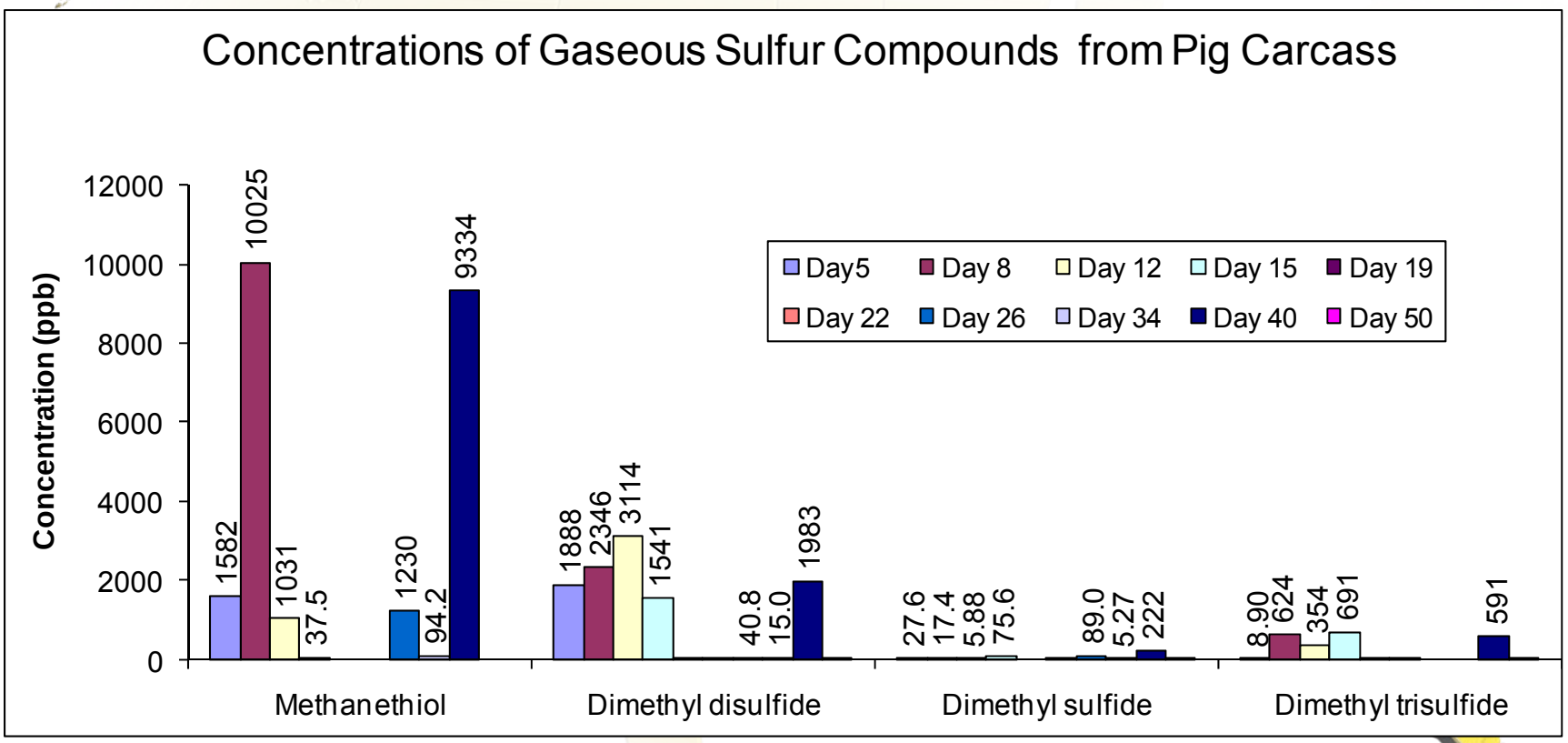
Calibration Curves of Selected Standard Compounds



Concentrations of VOCs for Day 5 Canister Samples Obtained from Sand, Soil, and Sawdust with Buried Pig Carcasses

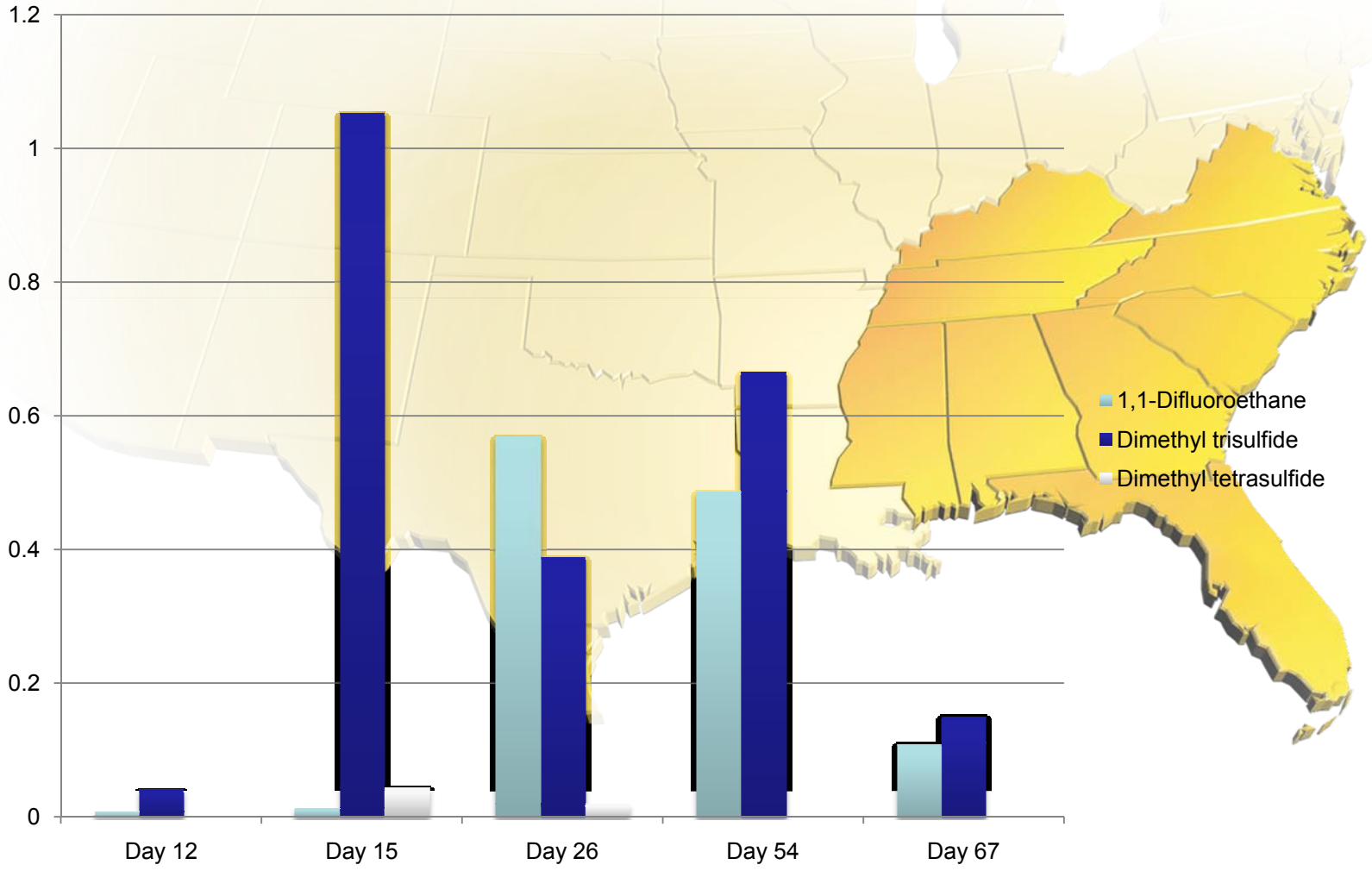
GC-MS of VOCs from Pig Carcass Buried in Soil (Gas Sampled Using Canisters)

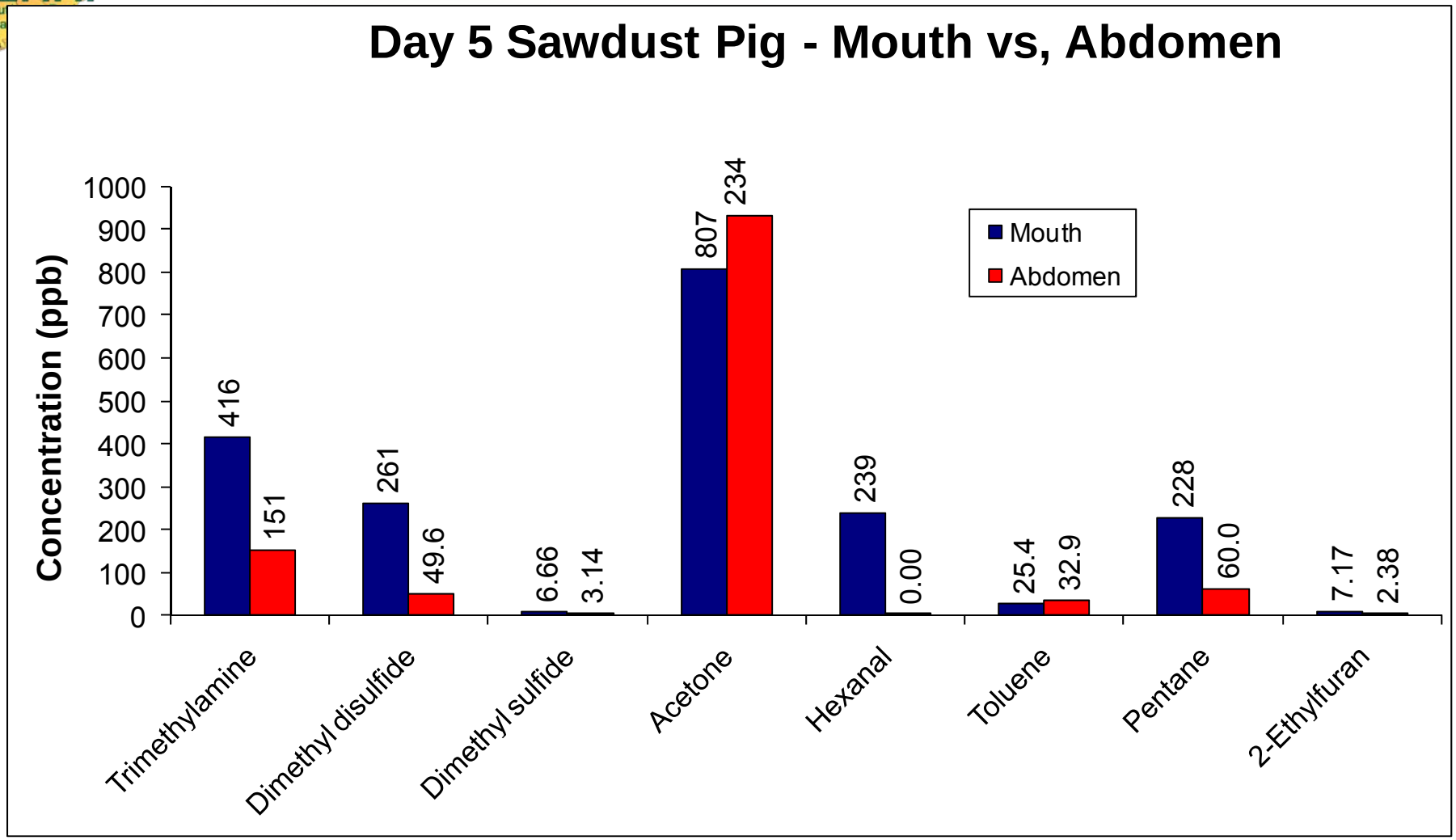




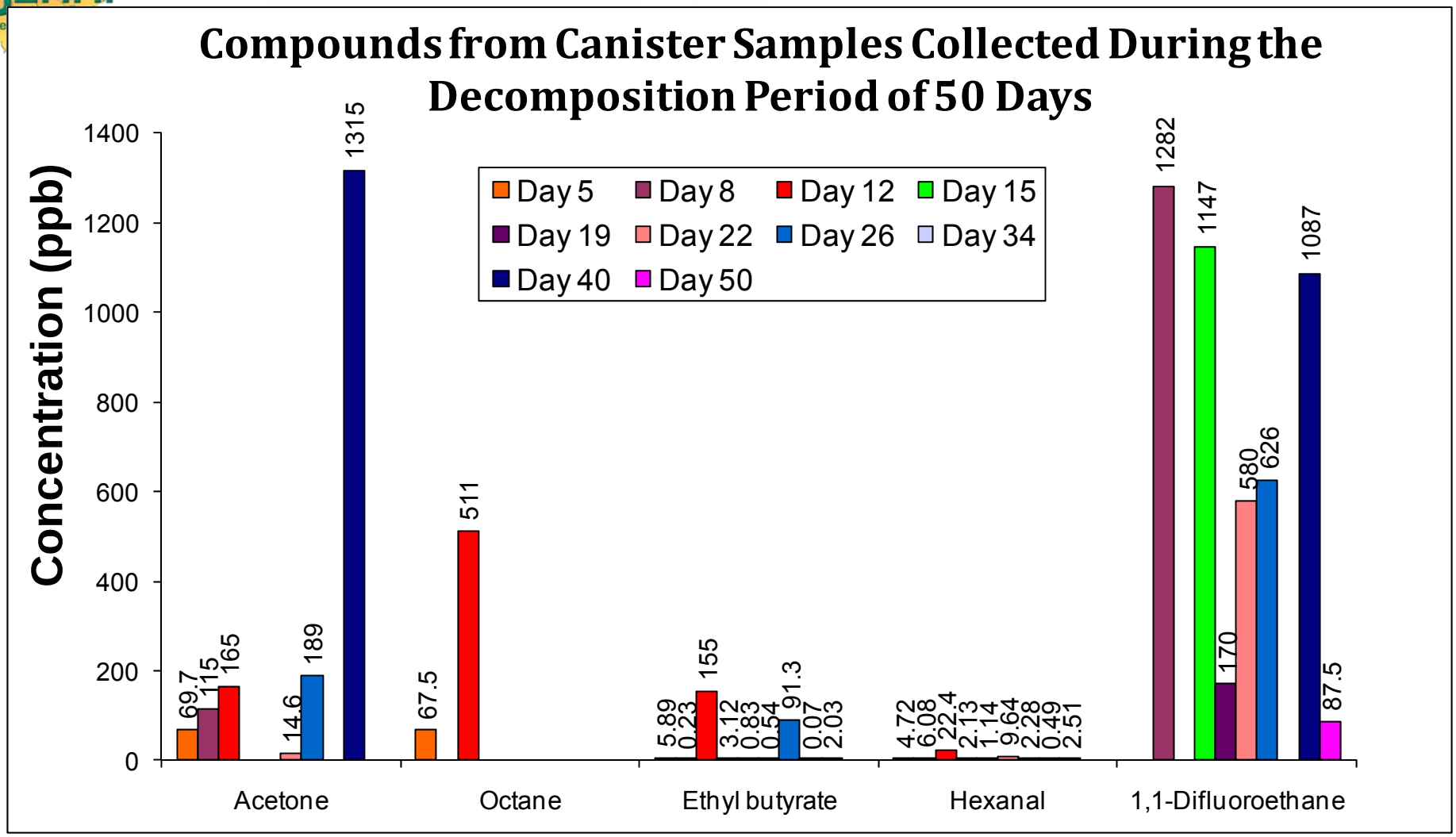
Concentrations of Sulfur Compounds from Pig Carcass Buried in Soil During 50 Days of Decomposition

Concentration Ratios of Compounds Relative to Dimethyldisulfide as a Function of Decomposition Time





VOC Concentrations from Day 5 Canister Samples Collected from the Mouth and Abdomen Areas of a Pig Buried in Sawdust



Concentrations of Volatile Organic Compounds from Pig Carcass Buried in Soil During 50 Days of Decomposition

Findings

- The advantage of using canisters for sampling VOCs from the decomposition of animal carcass is the ability to analyze volatile polar compounds like the carbonyls and alcohols in addition to the less polar compounds.
- GC-MS analysis showed the presence of sulfur compounds including methanethiol, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide; the higher molecular weight sulfur compounds were found in the later stages of decomposition.
- 1,1-Difluoroethane levels increased in the late stage of decomposition, possibly due to the reaction of fluoride from the bones with other organic metabolic by-products.
- This study will lead to environmentally acceptable means for the disposal of animal remains and the determination of post-mortem intervals in forensic investigation.

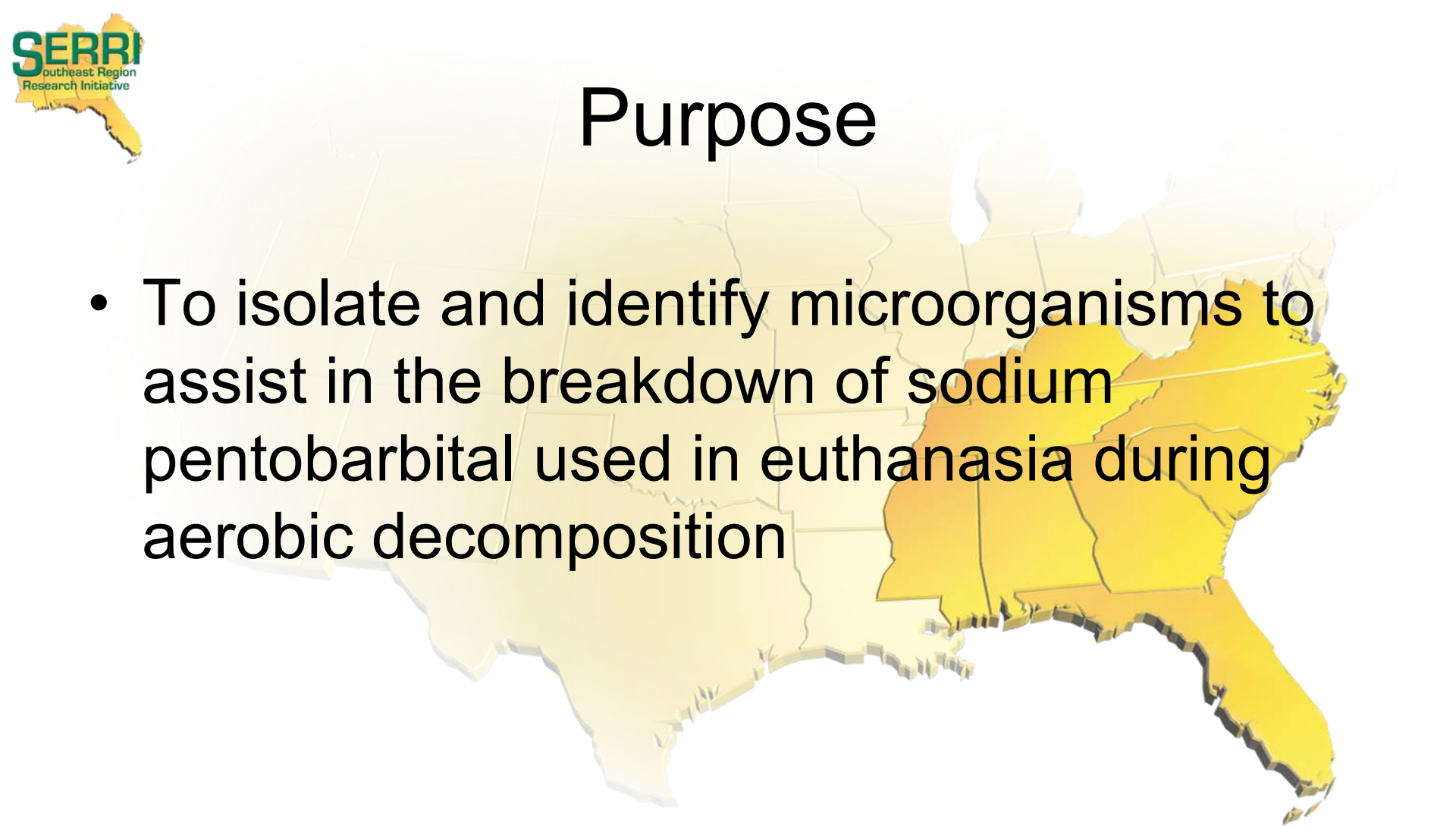
Isolation of Sodium Pentobarbital-Degrading Microorganisms

Mary B. Farone, Ph.D

- Diseased and dying animals are euthanized with barbiturate-containing solutions
- Uncovered carcasses disposed in landfills or used as donated carcass meat for carnivores in zoological institutions have resulted in barbiturate toxicity[1,2]
- Little data exists regarding drug residues in compost piles or potential risks to people, animals, or the environment

Purpose

- To isolate and identify microorganisms to assist in the breakdown of sodium pentobarbital used in euthanasia during aerobic decomposition



Methods and Current Status

1. Barbital Enrichment

Soil or stall manure/bedding sample

Minimal broth
+ 10 mM Glucose
37°C

Minimal broth + 8 mM Glucose
+ 0.1 mM sodium pentobarbital
37°C

Incrementally decrease glucose and increase sodium pentobarbital concentrations

Minimal broth
+ 1.0 mM sodium pentobarbital
37°C

2. Temperature Increase

Bacteria in 1.0 mM barbital

Incrementally increase incubation temperature in 2°C increments to 45°C

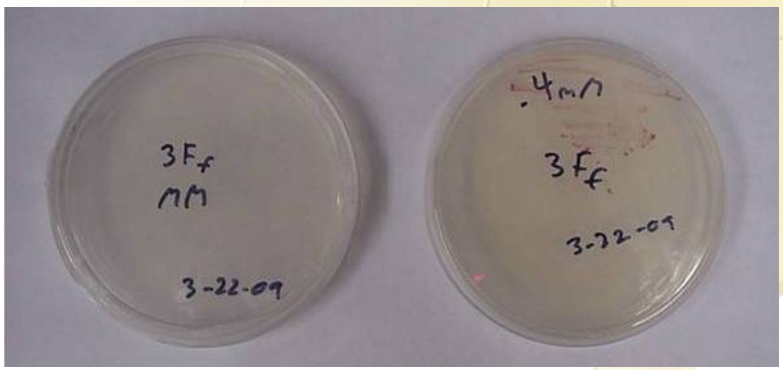
Bacteria growing in 1.0 mM sodium pentobarbital at 43°C

Culture Supernatants

ELISA analysis for barbital degradation

FINDINGS

- To date, 2 different bacteria have been isolated that show enhanced growth on barbital-containing media
- ELISA indicates potential barbiturate-degradation
  - currently being confirmed by MS/GC

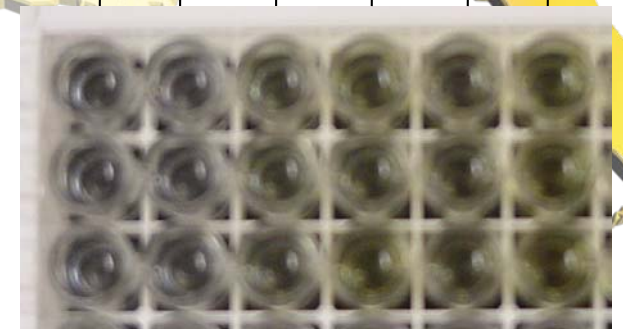


Minimal Media (MM) only

- No sodium pentobarbital
- Bacterial isolate 3Ff does not grow

Minimal Media + 0.4 mM barbital

- Bacterial isolate 3Ff grows indicating dependence on sodium pentobarbital



*Yellow indicates absence of barbiturates

Testing of Compost Runoff for Potential Pathogens

Anthony L. Farone, Ph.D.

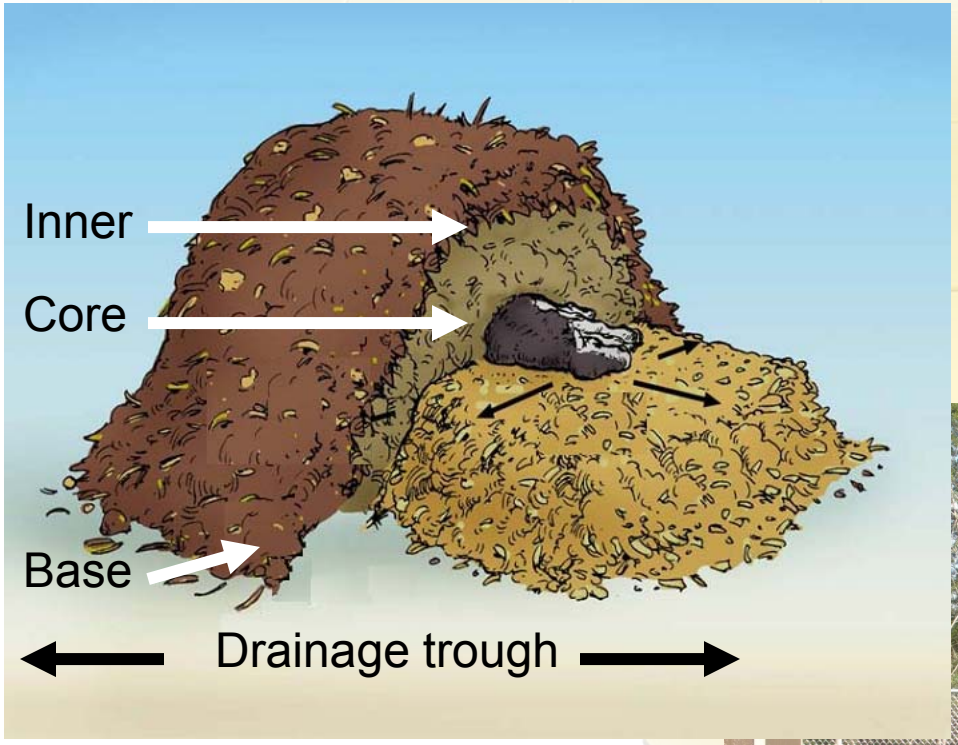
- Carcasses of composted animals may contain pathogenic microorganisms
- Source of the microorganisms is not always the result of a disease state but inhabitants of the respiratory, gastrointestinal, and genitourinary tracts of the animals
- Some microorganisms can survive outside of the host if composting temperatures are inadequate
- Regrowth can occur if conditions become favorable toward the end of composting

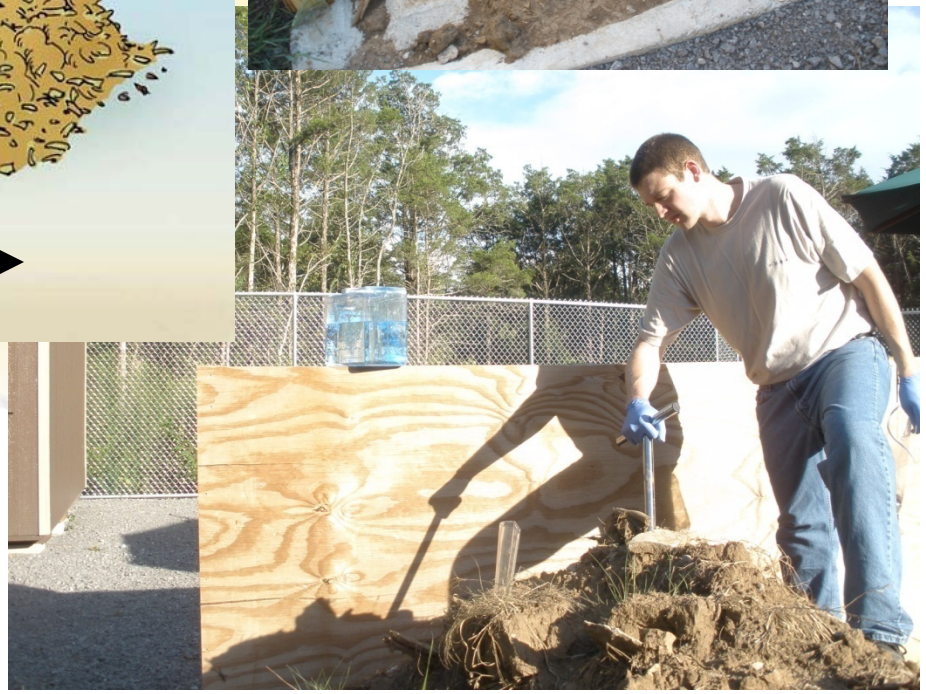
- Previous studies have indicated that pathogens such as *Salmonella spp.* and *Escherichia coli* O157:H7 can be inactivated at temperatures of 55-70°C
- However survival rates have varied from 9-59 days[3]
- Parvoviruses and enteroviruses have been eliminated by 28 days[4]

Purpose

- To perform risk assessment for each of the 3 burial media comparing the probability of harmful microorganisms being released into the environment

Sampling Areas

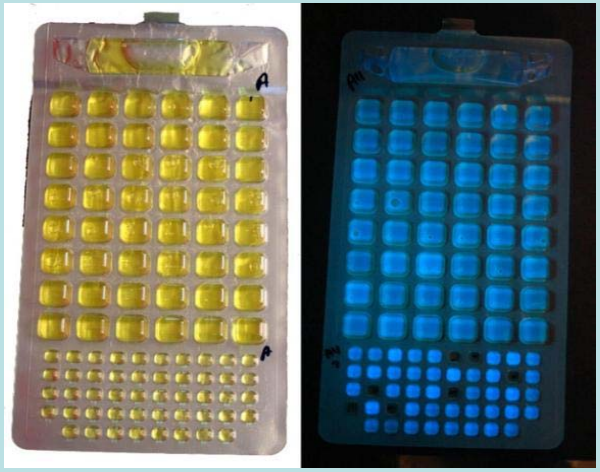




Methods

E. coli

Clear = No coliforms or *E. coli*

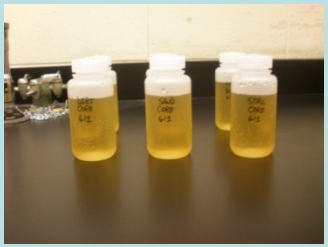


Yellow wells are positive for coliforms

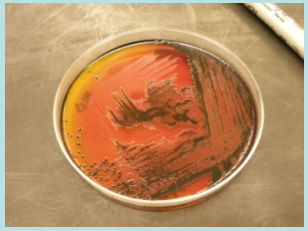
Blue wells are positive for *E. coli*

Salmonella

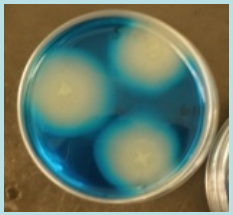
Buffered Peptone Water



Allows Salmonella to grow but inhibits other bacteria



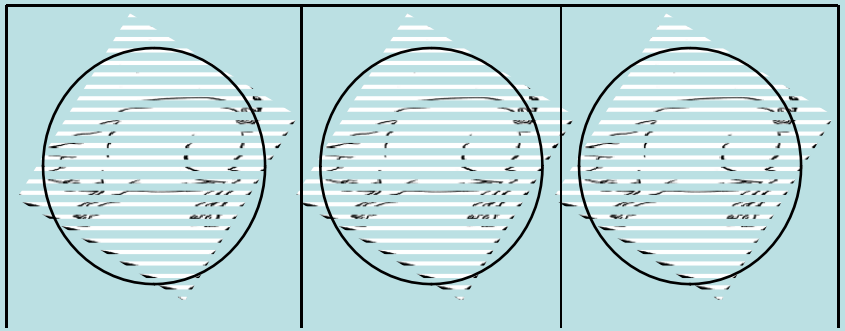
Presumptive *Salmonella* colonies are black



Rappaport-Vassiliadis medium and incubated at 41°C

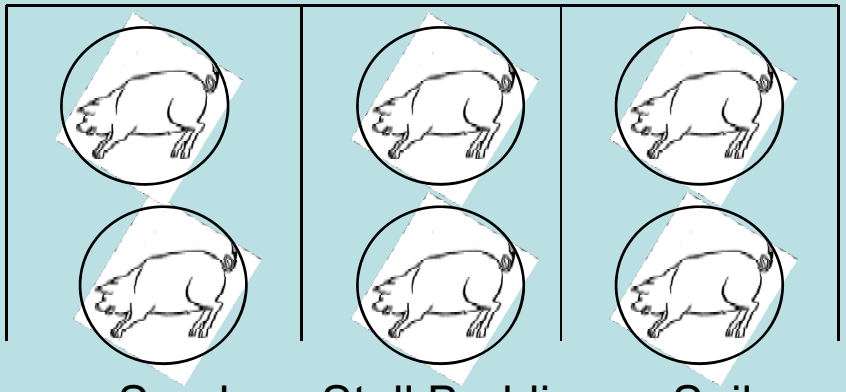
Experimental Set Up

Set Up A (begun 5-10-10)



Sand Stall Bedding Soil

Set Up B (begun 7-28-10)



Sand Stall Bedding Soil

- Sites were sampled 1-2 times per week
- For *E. coli* and fecal coliform evaluations, the **base samples** were tested to represent what would leach from the mound into the environment
- For *Salmonella*, base, inner, and core samples were tested for the presence of the bacterium

Presence of Fecal Coliforms

Fecal Coliform Presence – Set Up A

	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 8
Soil Base	-	ND*	+	+	+	+	+	+
Stall Base	+	ND	+	+	+	+	+	+
Sand Base	-	ND	-	+	-	-	-	-

* ND – not determined

Fecal Coliform Presence – Set Up B

	Day 0	Week 1	Week 2	Week 3	Week 4
Soil Base	+/-*	+/+	+/+	+/+	+/+
Stall Base	-/+	+/+	+/+	+/+	+/+
Sand Base	-/-	-/-	-/-	+/-	-/-

* Represents results for mound 1/mound 2

Presence of *E. coli*

Most Probable Number of *E. coli* per gram of compost – Set Up A

	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 8
Soil Base	0.00	ND*	0.00	0.00	0.00	0.00	0.00	0.00
Stall Base	2.03×10^4	ND	$>4.35 \times 10^5$	$>4.35 \times 10^5$	$>4.35 \times 10^5$	$>4.35 \times 10^5$	$>4.35 \times 10^5$	$>4.35 \times 10^5$
Sand Base	0.00	ND	0.00	0.00	0.00	0.00	0.00	0.00

* ND – not determined

Most Probable Number of *E. coli* per gram of compost – Set Up B

	Day 0	Week 1	Week 2	Week 3	Week 4
Soil Base	0.00/0.00*	0.00/0.00	0.00/0.00	0.00/0.00	$>4.35 \times 10^5$ /0.00
Stall Base	0.00/0.00	+//$>4.35 \times 10^5$	0.00//$>4.35 \times 10^5$	0.00/$>4.35 \times 10^5$	0.00/$>4.35 \times 10^5$
Sand Base	0.00/0.00	0.00/0.00	0.00/0.00	$>4.35 \times 10^5$ /0.00	0.00/0.00

* Represents results for mound 1/mound 2

Presence of *Salmonella*

Soil	Day 0	Week 2	Week 3	Week 4	Week 5	Week 6	Week 8
Core	-	-	-	-	-	-	-
Inner	-	-	-	-	-	-	+
Base	-	-	-	-	-	-	+

Stall	Day 0	Week 2	Week 3	Week 4	Week 5	Week 6	Week 8
Core	-	-	-	-	-	-	-
Inner	-	-	-	-	-	-	-
Base	-	-	-	-	-	-	-

Sand	Day 0	Week 2	Week 3	Week 4	Week 5	Week 6	Week 8
Core	-	-	-	-	-	-	-
Inner	-	-	-	-	-	-	-
Base	-	-	-	-	-	-	-

Summary of Pathogen Testing

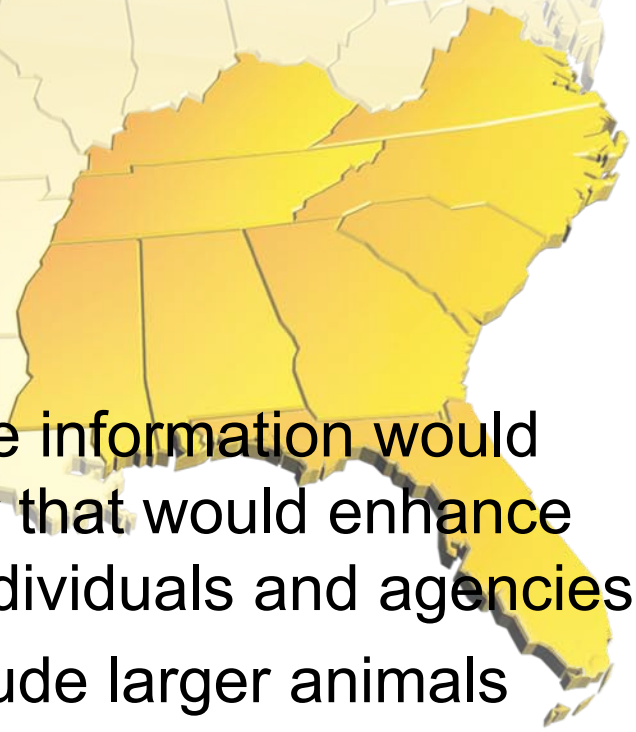
- Fecal coliforms persisted in both stall bedding and soil mounds throughout the sampling periods
- Only the stall bedding contained significant amounts of potentially disease-causing *E. coli*
- Sand was the best medium for preventing growth of bacteria
- No significant amounts of *Salmonella* were detected in these studies

Other Considerations:

- Significant rainfall events could contribute to the leaching of pathogens from the compost mounds into the environment
- The density of the compost material may play a role in the failure to reduce pathogens. The stall bedding material was the least dense of all of the media. This might facilitate the movement of bacteria throughout the compost mound
- Soil type can also vary regionally and different soils may be more or less conducive to bacterial growth

Collaborative Opportunities

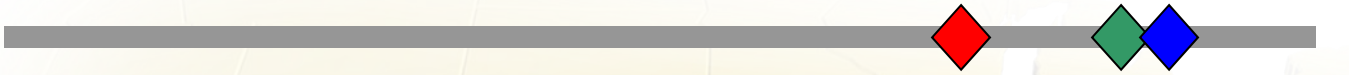
- The project is ongoing and assessment and training have not occurred yet
- Collaborations:
  - TEMA
  - UTK
  - Tn. Dept. of Agriculture
- Upon completion of the reports the information would need to be presented in a manner that would enhance broad distribution to concerned individuals and agencies.
- Phase 2 funding is needed to include larger animals such as horses



Project Timeline

Month	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Facility Construction															
Permits															
Bids															
Begin construction															
Road built															
Construction complete															
Aquire Decomposition Media															
Decompose Pigs															
200 pound pigs															
Excavate pigs															
50 pound pigs															
Excavate pigs															
Data Collection & Analysis															
Written Report															

Project Progress Summary



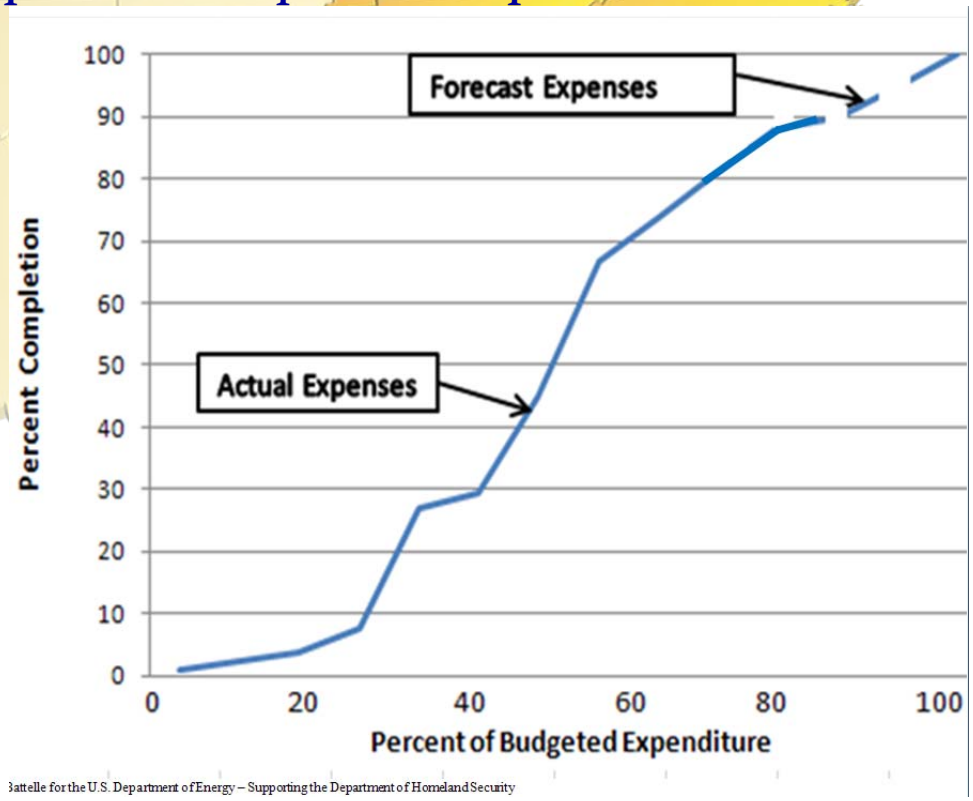
Budget Status: 82% of budget expended.

Technical Status: 70 % of project completed.

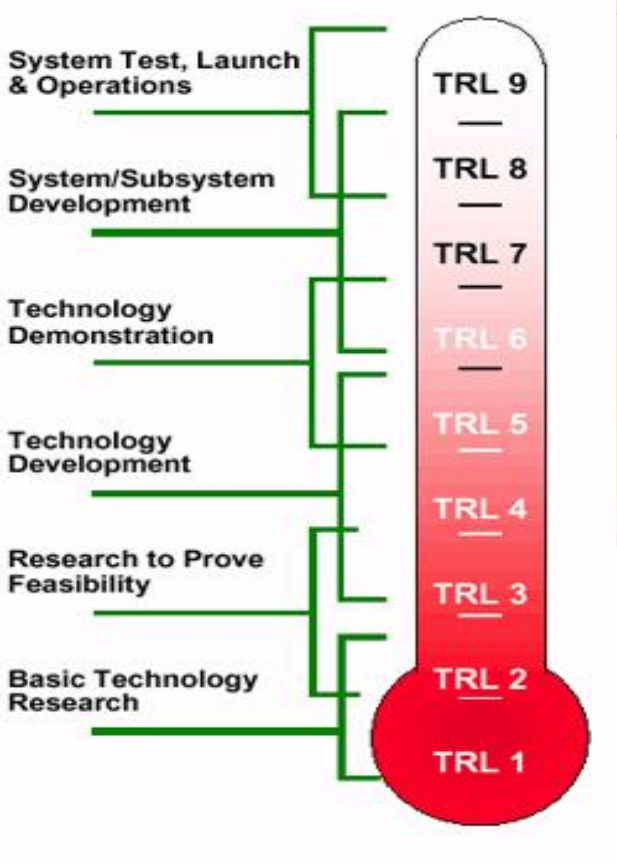
Schedule Status: 86 % of performance period completed.

- The progress was slow in the early stages due to construction of the test facility. Once that was completed and the equipment was purchased, the testing went quickly. The data analysis and report writing will be completed by the extension deadline.

- The chart shows spending at 82% while technical status is 70% and schedule status is 86%. This is due to construction and equipment costs being unequally distributed. Those onetime costs are now accounted for and the final expenses will be less through the project completion. We foresee that the project will be complete on time within budget.



Technology Readiness Level (TRL) Assessment



Technology	TRL
Chlorine Dioxide	7
Pentobarbital	3
Decomposition Gases	1

Summary & Conclusions

- Summary: discoveries and/or the capabilities/features of new product/tool developed.
  - Sand seems to be very effective in sterilization of runoff
  - Bacteria that decompose Pentobarbital
  - Chlorine dioxide is an effective surface disinfectant (anthrax)
  - Findings used to revise mass disaster protocols
- Unexpected developments encountered during the project (the positives as well as the negatives), issues, risks,
  - The monitoring equipment failed due to overheating
  - Loss of large animal disposal service in the middle Tennessee region significantly increased agricultural industry interest in the project.
- Plans to correct lack of progress if project is not on schedule (mitigation strategy)
  - Extension has been granted to complete phase 1 by the end of the year
  - Phase 2 (horse/cow) has been requested

Continued on next slide

Summary & Conclusions

- Explain any developments that may alter the anticipated outcome of the project
  - We see no problems from here on
- Plan of work for the next six months
  - Bacterial degradation of pentobarbital
  - Chlorine dioxide effectiveness on Anthrax spores
  - Continue work on bacterial degradation of pentobarbital
  - Isolate and characterize chemicals of decomposition in order to neutralize foul odors
  - Depends on grant extension
- Anticipated schedule or budget adjustments; changes in key personnel.
  - Dr. Haffner is leaving the university