Pentobarbital Testing and Clemson University Animal Co-Products Research & Education Center (ACREC) Update

Animal Co-Products Research & Education Center
CLEMSON UNIVERSITY

Annel K. Greene, Ph.D.
Center Director

Pentobarbital: Test Kit Review

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North American Renderers Association

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University of Georgia School of Veterinary Medicine

Overview of Project and Goals:

This project is an exploratory study to determine if commercially available rapid test kits designed for detecting barbiturates in human urine can be used to detect pentobarbital drugs in dead animals. If successful, the test kits would be recommended for renderers to test deadstock animals which may have been euthanized using pentobarbital.

Background

CLIA waived is based on the Clinical Laboratory Improvement Amendments (CLIA) passed by Congress in 1988. These amendments established the authority to enforce standards for certain laboratory testing. This was related to ensuring the accuracy and reliability of test results regardless of who performed the test.

CLIA waived tests are made to be simple and very easy to use with little to no chance for error.

As part of this, you will likely need to have your legal department look into CLIA waived for use in animal tissue testing and aspects related to rejection of an animal based on a CLIA waived test.

Reviewed Commercially Available rapid test kits

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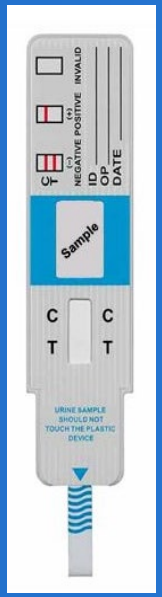
The majority of these test kits were designed for use in urine in humans with a few test kits for saliva and some for law enforcement use. The remaining were tests via high pressure liquid chromatography (HPLC) or gas chromatography (GC) methods.



We were looking for CLIA waived type products.

From this list, we chose 13 rapid test kits from eight different vendors for testing.

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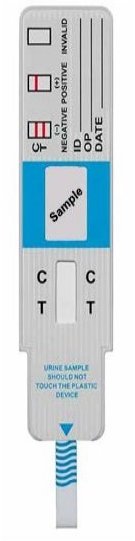
Examples – dipstick type test kits

Designed for testing barbiturates in human urine



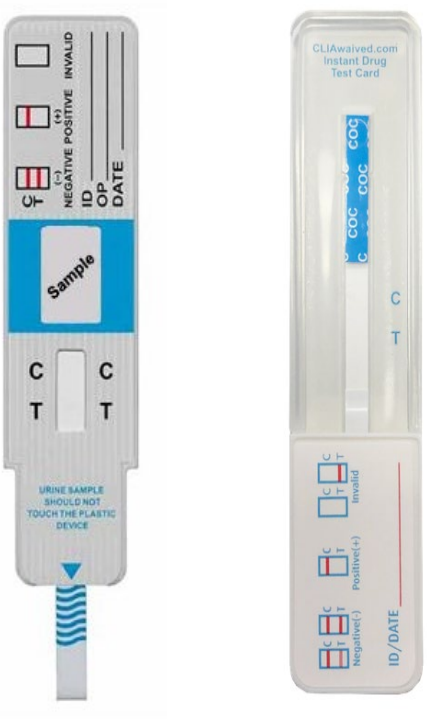
Designed for testing in urine

All of these kits work in a similar manner

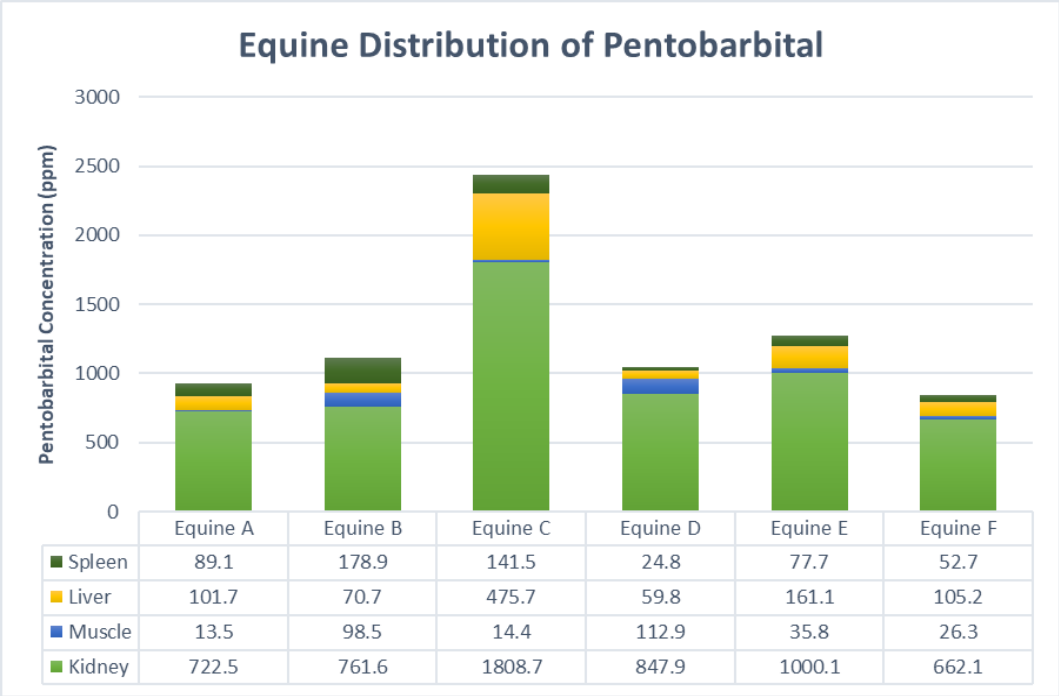
Collect a sample, dilute in water or buffer

Dip into a sample, allow to absorb up the strip

Read results



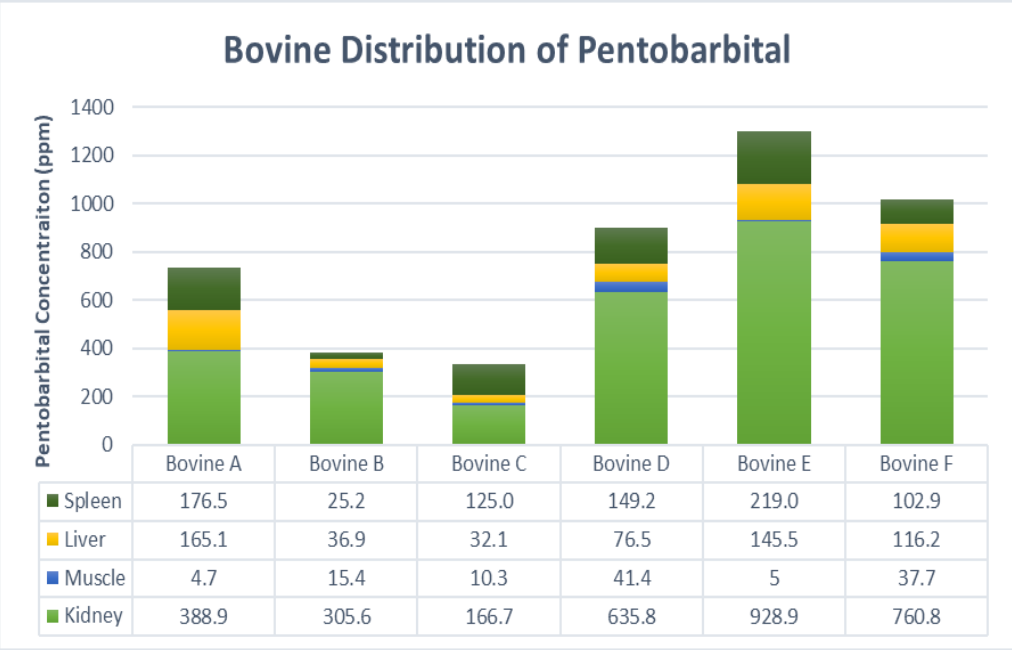
What is the target amount?



	Equine A	Equine B	Equine C	Equine D	Equine E	Equine F
Spleen	89.1	178.9	141.5	24.8	77.7	52.7
Liver	101.7	70.7	475.7	59.8	161.1	105.2
Muscle	13.5	98.5	14.4	112.9	35.8	26.3
Kidney	722.5	761.6	1808.7	847.9	1000.1	662.1

Pentobarbital concentrations found in liver, muscle, kidney and spleen from the sample population of equids euthanized with pentobarbital (from Tyrrel, Sept 2018)

What is the target amount?

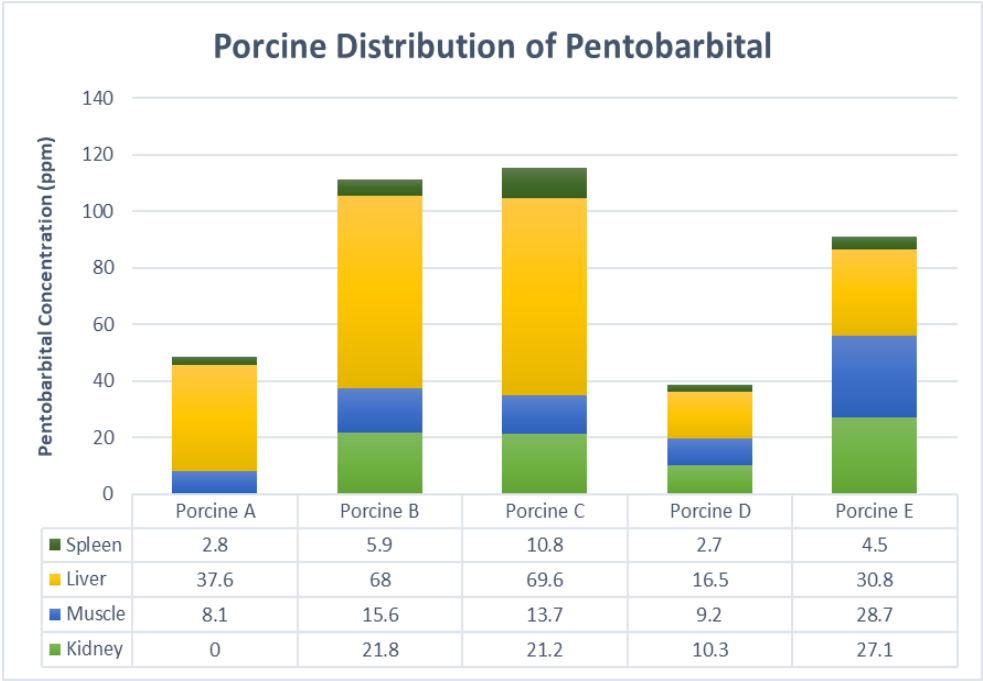


	Bovine A	Bovine B	Bovine C	Bovine D	Bovine E	Bovine F
Spleen	176.5	25.2	125.0	149.2	219.0	102.9
Liver	165.1	36.9	32.1	76.5	145.5	116.2
Muscle	4.7	15.4	10.3	41.4	5	37.7
Kidney	388.9	305.6	166.7	635.8	928.9	760.8

Pentobarbital concentrations found in liver, muscle, kidney, and spleen from the sample population of bovines euthanized with pentobarbital (from Tyrrel, Sept 2018)

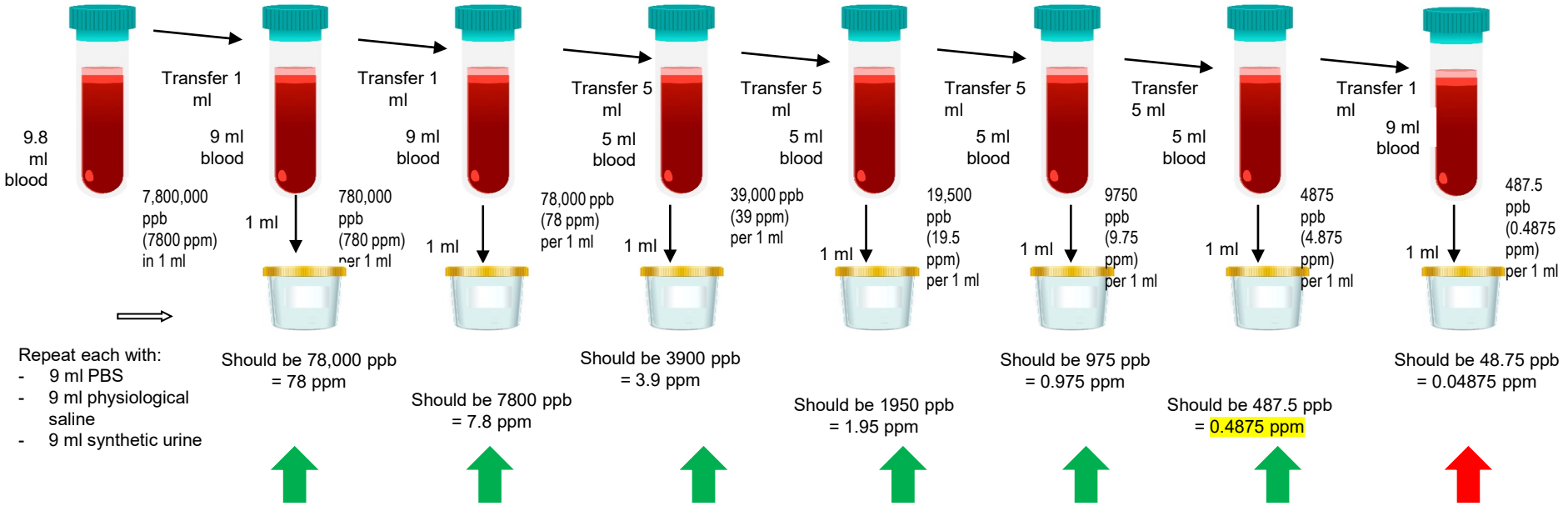
What is the target amount?

Pentobarbital concentrations found in liver, muscle, kidney, and spleen from the sample population of swine euthanized with pentobarbital (from Tyrrel, Sept 2018)



	Porcine A	Porcine B	Porcine C	Porcine D	Porcine E
Spleen	2.8	5.9	10.8	2.7	4.5
Liver	37.6	68	69.6	16.5	30.8
Muscle	8.1	15.6	13.7	9.2	28.7
Kidney	0	21.8	21.2	10.3	27.1

Validated test kits could detect down to 0.4875 ppm



Repeat each with:
- 9 ml PBS
- 9 ml physiological saline
- 9 ml synthetic urine

Should be 78,000 ppb = 78 ppm

Should be 7800 ppb = 7.8 ppm

Should be 3900 ppb = 3.9 ppm

Should be 1950 ppb = 1.95 ppm

Should be 975 ppb = 0.975 ppm

Should be 487.5 ppb = 0.4875 ppm

Should be 48.75 ppb = 0.04875 ppm

Samples collected

	Total Samples	Euthanized	Non-Euthanized
Equine	12	11	1
Bovine	21	2	19
Caprine	6	6	0

Testing Procedure

Cut a one-half inch square piece of haired skin (recommend use 1 inch square).

Placed in a sterile urine cup

Added 10 mls (2 tsp) of bottled water (Dasani™ brand)

Closed the lid on the urine cup

Swirled samples to thoroughly mix for one minute

Tested with four brands of test strips

Testing – storage at room temperature

0 hour	24 hour	48 hour	72 hour	96 hour

Results - Equine

	0 hour	24 hour	48 hour	72 hour	96 hour
Euthanized (11)*	All positive	All positive	All positive	All positive	All positive
Non-euthanized* (1)	Negative	Negative	Negative	Negative	Negative

* Samples collected from neck

Results - Bovine

	0 hour	24 hour	48 hour	72 hour	96 hour
Euthanized by UGA (1)*	Positive	Positive	Positive	Positive	Positive
Euthanized by local vet (1)**	Negative	Negative	Negative	Negative	Negative
Non-euthanized* (19)	All negative	All negative	All negative	All negative	All negative

* Sample collected from neck

** Sample collected between front legs

Results - Caprine

	0 hour	24 hour	48 hour	72 hour	96 hour
Euthanized by UGA (1)*	Positive	Positive	Positive	Positive	Positive
Euthanized by local vet (5)**	3 positive 2 negative	3 positive 2 negative	3 positive 2 negative	3 positive 2 negative	2 positive 3 negative

* Sample collected from neck

** Sample collected between front legs

All brands of test strips

Results were identical across all brands

Location, Location, Location

Selection of sampling location is critical

Want a highly vascular area; Suggest on neck near head

Tests

Inexpensive and rapid

- Total test time about 2-3 minutes
- Cost ranges from $1 to $3 each

Reports from Industry

Suggest sampling from fluid collected out of trucks

Suggest low levels associated with calving

Suggest the test strips are working very well

Further work

One of the FPRF member companies has graciously volunteered to do HPLC testing to measure exact concentrations of pentobarbital in the samples

This will be further evidence to support the results of this study

ACREC Update

Animal Co-Products Research & Education Center
CLEMSON UNIVERSITY

Dr. Kevin Finneran

RENDERING CO-PRODUCTS AS ELECTRON DONORS FOR SUBSURFACE REMEDIATION: Industry White Paper on the Use of Co-Products as Electron Donors and Associated Marketing at National Conferences

Small project to prepare a white paper to distribute at the National Groundwater Association and other conferences for the purpose of marketing animal co-products in environmental remediation.

Dr. Kevin Finneran
Professor
DEPARTMENT OF ENVIRONMENTAL ENGINEERING AND EARTH SCIENCES

Dr. Kevin Finneran

This work will build on Dr. Finneran's successful previous research which demonstrates animal co-products and especially the lower value products, have great potential for groundwater remediation.

He has proven in real world applications that rendered co-products can stimulate trichloroethylene and hexavalent chromium biodegradation. The co-products serve as electron donors in the cleanup process.

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Every animal co-product tested stimulated environmental remediation much better than any commercially available electron donor (most of which are derived from soybeans).

The lower value products the industry produces work just as well as some of the higher value products.

Trichloroethylene (TCE) is a major contaminant and is found at more than 75% of contaminated sites.

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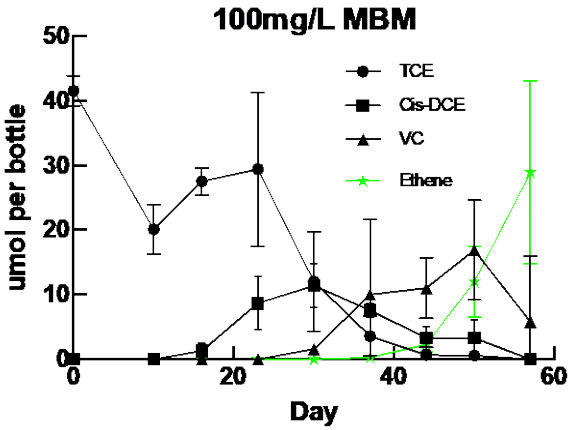

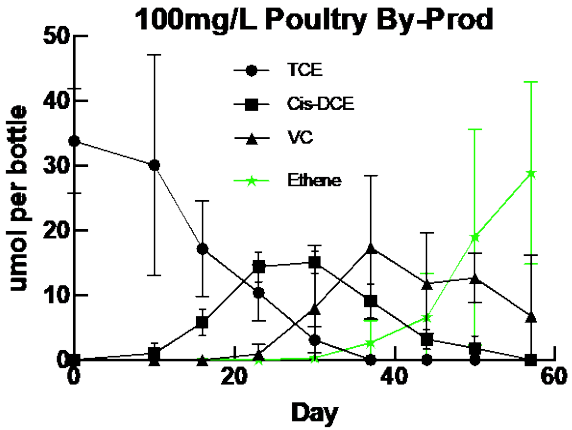

TCE reduction in MBM amended (left) and Poultry By Product Amended (right) incubations. Both are equivalent or better than soybean oil based electron donors.

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Dr. Finneran has a patent pending on the technology and his working to move this technology to market.

This will expand markets for the rendering industry with a high value product from some of the lower valued rendering products.

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On-Site Demonstration of Electrocoagulation Treatment of Rendering Wastewater

Developed electrochemical cells that can be used to treat wastewater instead of DAF

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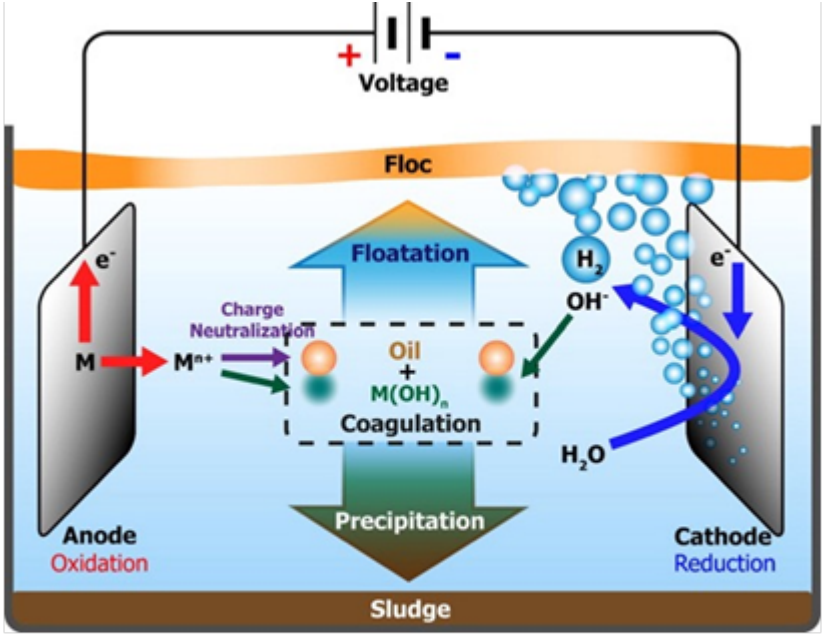

Figure 1. A schematic showing the mechanisms of fats and solids removal in EC.

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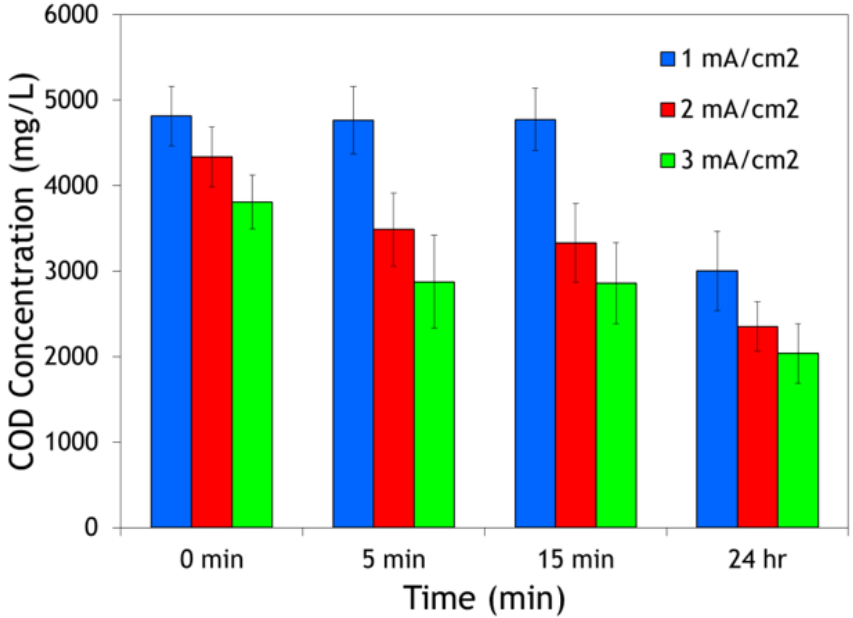

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Will deploy their unit at rendering plants and monitor for performance

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Dr. Julie Northcutt & Dr. Paul Dawson

Instructional Video on Environmental Sampling for Pathogenic Microorganisms in Rendering Plants

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Environmental monitoring program training program will be prepared as a video presentation for use in educational programs by APPI and individual companies

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Dr. Rhett Smith

Low-Value Rendering Cements for Affordability and Commercialization

Dr. Rhett Smith
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DEPARTMENT OF CHEMISTRY

Dr. Rhett Smith

Using brown grease to make a cement material and optimizing the mechanical properties

Testing samples for mechanical strength, Recyclability, and chemical resistance

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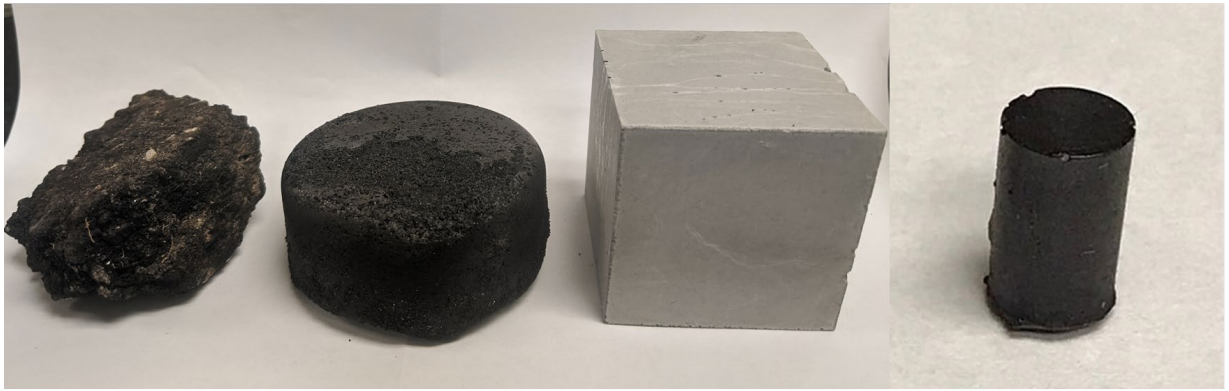

Asphalt Oleic Acid-Sulfur Asphalt Portland Cement Brown Grease Cement

Dr. Rhett Smith
Professor
DEPARTMENT OF CHEMISTRY

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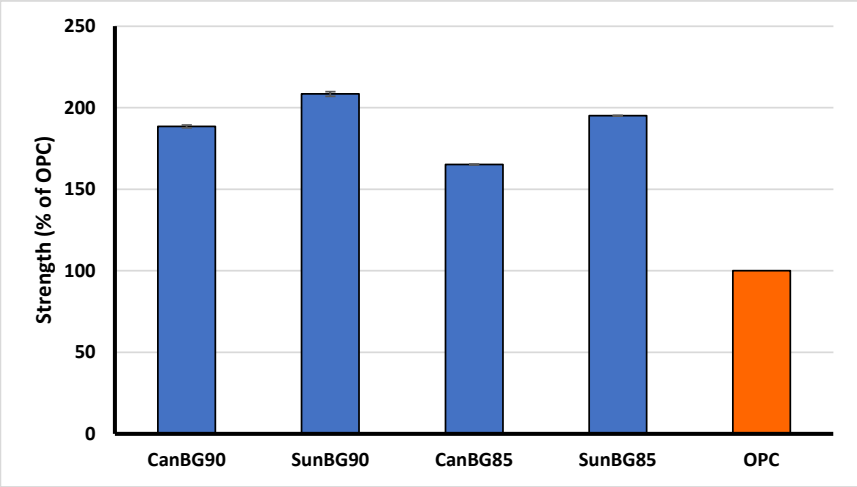

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Compressive strengths of brown grease-plant oil-sulfur cements exceed that of ordinary Portland cement (OPC). CanBG cements are made of canola oil, brown grease and sulfur. SunBG cements are made of sunflower oil, brown grease and sulfur. The number indicates the wt% sulfur used in the composite.

Dr. Rhett Smith

Packaging and Shipping Goods from Low-Value Rendering Products: Plastics and Pressure

Dr. Rhett Smith
Professor
DEPARTMENT OF CHEMISTRY

Dr. Rhett Smith

The goal of this proposal is to use low-value fats (LVFs) such as brown grease as starting materials to develop packaging materials that outperform existing technologies

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Dr. Annel Greene

Analysis of Rendered Meals for Boron

Ms. Dana McCurdy
PhD Graduate Student
DEPARTMENT OF ANIMAL & VETERINARY SCIENCES

Boron

Boron is an important nutrient involved in a large number of health-related processes in the body including prevention of arthritis, enhancing hormonal function, ensuring embryonic development, maintaining proper cell membrane function prevention of osteoporosis and in formation of strong bones.

Boron is involved in lowering congestive heart failure, reducing plasma lipid levels, improving brain function and also in fighting fungal infections.

Proper boron levels are associated with lower risk of lung, cervical and prostate cancer and even ability to inhibit progression of prostate cancer.

Boron

In a research meeting with poultry industry personnel, it was mentioned there are currently problems with chicken bones being very soft and high incidence of necrosis in the head of the femur.

Could the change to vegetarian diets could be related to a boron deficiency?

Boron

Although there is information about the importance of boron for bone health in poultry, there is no literature about boron content in rendered animal meals.

This project is an exploratory project to determine if rendered animal co-products contain boron.

Dr. Daniel Whitehead

Materials for Removal of Metal and Inorganic Contaminants from Rendered Fat

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To remove metals for renewable diesel production.

Metals are limiting sales of animal fats into renewable diesel market and the industry is seeking a rapid metal-removal system

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Screen methods for removal of metal ions and other inorganics from spiked fat samples

Assess adsorption kinetics study

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Minimizing Antioxidant Use in Rendered Products via Artificial Intelligence

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This project assembles expertise in analytical chemistry, antioxidant chemistry, and artificial intelligence to promote the discovery of novel, high-performance antioxidant blends for rendered fat. The project will establish a working database of antioxidants and their properties by means of a combination of data mining and experimentation.

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This database will then be used to develop and train an Artificial Neural Network (ANN) that can be leveraged to uncover hitherto unknown antioxidant candidates and synergistic combinations thereof for use in the rendering and pet food industry.

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While the system will enable predicting the most efficient combinations of currently available antioxidants to preserve rendered animal fat, it will ultimately be able to identify new components to boost antioxidant synergism based on chemical interactions.

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This information will provide the basis to rationally lower the concentrations used and provide much more objective guidelines to either supplement or replace current antioxidant formulations.

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Thank you very much!

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