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fast breaking papers - 2010

February 2010

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Michael S. Filigenzi talks with *ScienceWatch.com* and answers a few questions about this month's Fast Breaking Paper Paper in the field of Agricultural Sciences.

Article Title: Diagnostic determination of melamine and related compounds in kidney tissue by liquid chromatography/tandem mass spectrometry

Authors: **Filigenzi, MS**;Puschner, B;Aston, LS;Poppenga, RH

Journal: J AGR FOOD CHEM, Volume: 56, Issue: 17, Page: 7593-7599, Year: SEP 10 2008

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SW: Why do you think your paper is highly cited?

The work done by my co-workers and I on the analysis of melamine came in response to the pet food poisoning incident in 2007. This incident illuminated previously unknown issues regarding melamine adulteration of Chinese pet food additives and the toxicity of melamine and its analogs.

An incident involving melamine's use as an adulterant in infant formula in China in 2008 resulted in a major increase in research on melamine, particularly among Chinese researchers. Our method was the first published method for the detection of melamine and its three analogs (ammeline, ammelide, and cyanuric acid) in tissue samples, and it's therefore commonly referenced in the many subsequent articles involving melamine analysis.

SW: Does it describe a new discovery, methodology, or synthesis of knowledge?

Our article describes a method for the analysis of melamine in kidney tissue for use in the diagnosis of melamine poisoning. Novel aspects of this method included the ability to detect melamine and its analogs in a single analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and the ability to detect melamine and cyanuric acid that are present individually in the kidney tissue as well as when combined into melamine cyanurate. This method is also capable of detecting melamine and its analogs in archived paraffin-fixed tissue.

SW: Would you summarize the significance of your paper in layman's terms?

Melamine and several related compounds can cause sudden kidney failure and death in animals (particularly dogs and cats) which consume them. In 2007, thousands of pets died from eating pet food which contained these toxins. It was later determined that melamine was intentionally added to one of the food ingredients in order to make it look as though that ingredient had much more protein in it than it really had.

We developed a method which can determine when an animal has died from eating melamine and/or its related compounds. This will make it much easier to contain any future outbreaks of pet illness and death due to melamine poisoning.

"Although this was a pet food incident, it tested the same systems our country has in place for handling such problems in human food."

SW: How did you become involved in this research, and were there any problems along the way?

We became involved in this research at the beginning of the 2007 incident involving melamine adulteration in pet food. Our group is part of a veterinary diagnostic laboratory so when animals suddenly began dying of what was then an unknown cause, we were involved in trying to determine what the causative agent was.

Once it was determined that melamine and its analogs were the culprits, we needed a method to diagnose this poisoning on a post-mortem basis. This resulted in the development of the method we reported in our article.

We encountered a number of challenges during the development of this method. Finding a high performance liquid chromatography (HPLC) column that could retain all of the analytes was the first issue. These compounds are all quite polar, which often means that the required mobile phase can't be used with a mass spectrometer. We were fortunate in having a column on hand that gave us the necessary retention of the analytes using a mobile phase that was "mass spec-friendly."

A second challenge came in the sample preparation step. Other researchers had shown that the melamine and cyanuric acid present in kidney tissue were combined into melamine cyanurate crystals. These crystals are insoluble in most common laboratory solvents. We experimented with several mixtures of solvents before finding one that reliably separated melamine cyanurate into its two constituents.

Finally, we hoped to be able to provide estimates of the concentrations of melamine and cyanuric acid present in the kidney tissue. Quantitative analysis is always tricky when using LC-MS/MS, particularly in complex matrices such as kidney tissue. The use of isotopically-labeled melamine and cyanuric acid enabled us to provide quantitative data using this method.

SW: Where do you see your research leading in the future?

Because of the aforementioned problem with melamine adulteration of baby formula, there has been an explosion of interest in these compounds, their toxicity, their pharmacokinetics in different food animals, and in preventing future instances of food adulteration. Our method and others similar to it will be used to support all of these areas of research.

Future work may also involve retrospective studies to identify past incidents of food tampering. Our lab has participated in studies involving analysis of archived paraffin-fixed kidney tissues in Korean dogs in

2003 (Yhee, *et al*., *Vet Pathol.* 46[2]: 348-54, 2009 Mar; and in Iberian piglets in 2003 – 2006 (Gonzalez, *et al., J. Vet. Diagn. Invest*. 21[4]:558-63, 2009 Jul).

In both cases, we were able to detect melamine and its analogs in fixed tissue that had been stored for several years. It was concluded that the deaths of animals in both instances were likely due to adulteration of their feed with melamine. As researchers become more familiar with the symptoms of melamine poisoning, we may see more such retrospective studies identifying past incidents of feed adulteration using melamine.

SW: Do you foresee any social or political implications for your research?

The pet food adulteration incident of 2007 was a nightmarish situation in several regards. All anyone knew at its outset was that pets were dying in significant numbers from an unknown poison present in many brands of commercial pet food.

"The globalization of our food supply has made it increasingly difficult to assure consumers that their food is safe"

Getting this situation figured out in a short time frame was a very difficult endeavor. I would be remiss if I did not mention the intense level of coordination between our group and the USFDA, other state veterinary diagnostic labs, and private labs during this crisis. This cooperation between groups was crucial in the rapid identification of the toxins, their mode of action, and in the development of methods to detect them.

Although this was a pet food incident, it tested the same systems our country has in place for handling such problems in human food. Everyone involved in this incident learned a lot from it and we are now much better prepared to handle future incidents of food contamination or adulteration.

The political and social implications of melamine adulteration in food have been far more prominent in China. As of this writing, two people have been executed and several others jailed for their roles in the sale of infant formula adulterated with melamine.

Consumer confidence in the Chinese dairy industry was severely shaken as the prevalence of melamine adulteration became known and as many thousands of infants were hospitalized with kidney stones. Many other countries which import milk products from China were also heavily affected by this.

The methods developed by our group and by others involved with the pet food incident enabled officials around the world to rapidly determine the extent of the adulteration. These methods also enabled officials in our own country to rapidly survey imported foods to insure that our own citizens were not at risk.

In both of the above instances, illicit adulteration of a food product in one country had severe implications for individuals in many other countries. The globalization of our food supply has made it increasingly difficult to assure consumers that their food is safe. Rapid, accurate analytical methods such as ours will be increasingly important in maintaining the safety and security of the food we eat.

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SPECTROMETRY; CYANURIC ACID; METABOLITE MELAMINE; CYROMAZINE; ELECTROSPRAY; SEPARATION; PRODUCTS; SAMPLES; URINE; WATER; CATS.

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