5 Melamine

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Melamine is an organic compound with a triazine skeleton (Figure 5.1A). It contains 67% nitrogen by mass and is combined with formaldehyde to make melamine resin used in the manufacture of formica, melamine dinnerware, laminate flooring, and dry erase boards. Melamine also combines with cyanuric acid (Figure 5.1B) and related compounds to form melamine cyanurate crystals (Figure 5.1C), which have been found as contaminants or biomarkers in cases of protein adulterations. Melamine is sometimes illegally added to food products and feed ingredients to increase the apparent protein content. In proximate analyses of crude protein using the Kjeldahl method, protein levels of feed ingredients are estimated by measuring the nitrogen content and multiplying this by a factor of 6.25, thus the protein levels of poor quality ingredients can be intentionally increased by adding nitrogen-rich compounds such as melamine. In incidents that caused global concerns about food safety in 2007 and 2008, melamine has been intentionally added to animal feed or food products for humans (such as powdered milk or infant formulas) causing severe kidney damage to children and pets poisoned by melamine-adulterated products.

Method of detection

The U. S. Food and Drug Administration and the Japanese Ministry of Health, Labor and Welfare have issued methods based on liquid chromatography-mass spectrometry (LC/MS) detection of melamine and cyanuric acid after hydrophilic interaction liquid chromatographic (HILIC) separation.

The existing methods for melamine determination using liquid chromatography – mass spectrometry (LC/MS) after solid phase extraction are often complex and time consuming. Improvements using electrospray ionization methods coupled with mass spectrometry allow a rapid and direct analysis of samples with complex matrices where the native liquid samples are directly ionized under ambient conditions in their original solution.

Ultrasound-assisted extractive electrospray ionization mass spectrometry (EESI-MS) has been developed for a rapid detection of melamine in untreated food samples. Ultrasound is used to nebulize the melamine-containing liquids into a fine spray. The spray is then ionized by extractive spray ionization and analysed using tandem mass spectrometry (MS/MS). An analysis requires 30

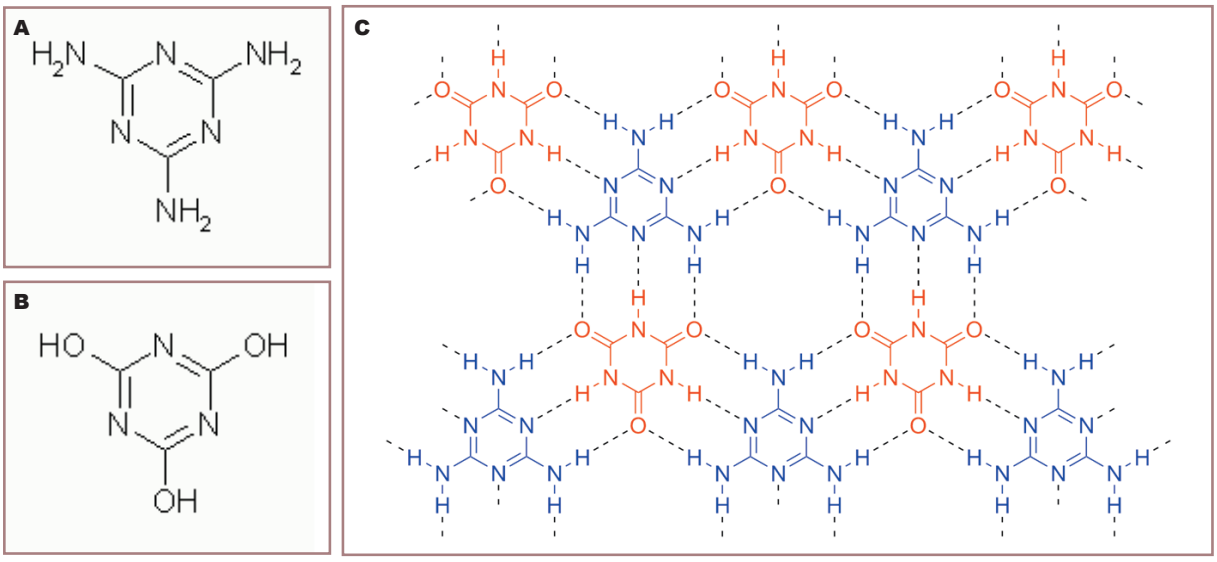

Figure 5.1. Chemical structures of (A) Melanine, (B) Cyanuric Acid and (C) Melamine Cyanurate Crystals

seconds per sample. The limit of detection of melamine in milk for example is a few nanograms of melamine per gram.

A simpler instrumentation and a faster method by using a low-temperature plasma probe to ionize the samples and mass spectrometry technique now allows high-throughput analysis of melamine traces in complex mixtures.

Toxicity

Melamine is an eye, skin, and lung irritant. If swallowed or inhaled it goes to the bloodstream and ultimately finds its way into the kidney tubules. In the kidney, melamine and cyanuric acid are concentrated forming large numbers of crystals which can deposit in and damage the cells lining the kidney tubules causing the kidney to malfunction. Renal failure has been observed in humans, other mammals, and fish.

Tissue (plasma, muscles, kidneys, liver, and gills) deposition of melamine and cyanuric acid following continuous voluntary feeding was observed in rainbow trout (Liu et al. 2014). Upon withdrawal, the melamine and cyanuric acid concentration in the tissues decreased exponentially. It was also noted that melamine is converted into cyanuric acid in trout. Muscles residues and renal crystals also formed in catfish and rainbow trout given melamine and cyanuric acid, persisting for weeks after the single dose (Reimschuessel et al. 2010). Studies have also shown a much slower elimination of melamine and cyanuric acid from rainbow trout body (Xue et al. 2011). Crystal formation in trout kidney was found to be both dose and time dependent (Pacini et al. 2014). Even after six weeks of withdrawal, the crystals persisted in fish that received the high dosage. Furthermore, crystals within multifocal hemocytic granulomas in the antennal gland tubules and peritubular hemal sinuses of penaeid shrimps that were very similar to melamine-cyanuric acid-induced crystals in mammalian kidney with melamine induced renal failure have also been observed in shrimps fed melamine-contaminated feed (Lightner et al., 2009).

Summary and recommendations

Melamine is an adulterant that can be added to feed ingredients for aquafeeds to artificially inflate the apparent protein content. Together with cyanuric acid, it has been found that crystals formed from melamine and cyanuric acid can cause kidney damage in mammals, fish, and shrimp. If in doubt of the source and quality of feed ingredients and aquafeeds, samples should be submitted for melamine and cyanuric acid analysis. Their presence in feed ingredients and aquafeeds are biomarkers for contamination, adulteration or intentional addition to increase crude protein levels. The United Nations' Codex Alimentarius Commission has set the maximum amount of 1 mg/kg melamine in powdered infant formula and 2.5 mg/kg in other foods and animal feed. While not legally binding, the recommended levels can serve as basis for banning the importation of products with excessive levels of melamine.

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