Review Article

A Comprehensive Review on Melamine: Insights into Risks and Detection Techniques

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Received: 14-04-2024; **Revised:** 27-06-2024; **Accepted:** 08-07-2024; **Published on:** 15-07-2024.

ABSTRACT

It is well known that milk has a high nutritional content, making it an ideal nourishment for babies, kids, and adults. Regrettably, milk adulteration is a major problem all throughout the world. A nitrogen-rich substance called melamine is added to milk to artificially raise the protein content of milk and dairy products. Milk, milk powders, chocolates, instant coffee, eggs, pet meals, raising agents, and other items have been linked to melamine. Melamine contamination is one of the biggest hazards to food safety for consumers. In September 2008, news broke of a significant food safety problem in China, an estimated 300,000 Chinese newborns and young children were impacted by renal and urinary tract consequences, including kidney stones, with six recorded deaths. For the purpose of accurately detecting and quantify melamine contamination several analytical methods are required. The amount of melamine can be detected from nanogram to microgram level using different analytical techniques such as mass spectroscopy, enzyme- linked immunosorbent assay (ELISA), high performance liquid chromatography (HPLC), and capillary electrophoresis. On the other hand, melamine finds widespread industrial use in reaction with formaldehyde to make several polymers such as plastic, laminates, paints, adhesives, and tensile finishers.

Keywords: Melamine, milk adulteration, infant formula, pet food, analytical techniques.

INTRODUCTION

This review article aims to provide a comprehensive understanding of melamine, its sources, and potential hazards to human health and the environment, as well as an overview of various analytical techniques used for its detection, to facilitate better management and regulation in relevant industries.

Melamine, a white crystalline powder that crystallizes as nitrogen-filled crystals, is a heterocyclic aromatic compound with the formula $C_3H_6N_6$. It is slightly soluble in water. Melamine is widely used in the production of sheets, plastic materials, coatings, adhesives, commercial filters, and some types of containers. The misuse of melamine has resulted in an excess of nitrogen, particularly in food, it has been illegally added to food products to increase their apparent protein content. The quantity of nitrogen increases when the melamine level of foods in milk and its derivatives, particularly milk powder and infant formula is raised. Melamine is not digestible and can lead to serious health issues when ingested in large amounts. The fraudulent addition of melamine to food products has been a significant concern in food safety, resulting in renal failure, kidney stones, and other health issues leading to various incidents and regulatory actions worldwide. However, in 2007 veterinary scientists found that melamine contamination in pet food was the reason for the hundreds of pet deaths. This finding brought melamine into the public eye. A significant pet food recall that affected both the US and Canada was linked to melamine. Certain imported cereal-based pet food ingredients (e.g. Wheat flour, wheat gluten, corn flour, and rice) protein concentration was deliberately adulterated with melamine to boost their total nitrogen content (melamine contains 66.6%N) by weight. In 2008 high melamine amounts were found in tainted Chinese baby formula, due to melamine contamination in baby formula and related dairy products, around 51,000 newborn and young children in China were admitted to hospitals for urinary issues, potential renal tube obstructions and potential kidney stones. There have been six verified newborn fatalities[1].

Subsequently, it was discovered that melamine could be present in protein powders, frozen desserts, cereal items, candies, cakes, and biscuits, plus several processed foods. Following that, it was discovered that several Chinese-made nondairy goods were melamine-contaminated. Among these goods were ammonium bicarbonate, dried whole eggs, fresh hen eggs, animal feed, and animal feed ingredients (nondairy creamer). Melamine is not a natural product and is not approved for direct addition to food or feed; however, it is approved for use as part of certain food contact substances. Melamine, sometimes referred to as tripolycyanamide, is an industrial chemical used to make melamine resins, which are used in plastics, laminates, and glues as shown in figure 1[2].

There are several ways that melamine can get into food: I) resins containing melamine that are utilized in food packaging. II) melamine migration happens when high temperatures and different food solvents like ethanol (alcoholic beverages), acetic acid (pickles and vinegar), or melamine formaldehyde cups containing coffee, orange juice, fermented milk, or lemon juice are applied to kitchen equipment. III) Melamine migration through components of adhesives and coatings used in food contact materials and labeling. IV) cyromazine residues from applications as a veterinary medication, insecticide (in plant products such as animal and poultry feed), and herbicides. V) resting hygienic solutions that are used as a disinfectant and sanitizer on food processing equipment and packaging materials that eventually breakdown into melamine by mixing trichloromelamine and dichloroisocyanurate. VI) the usage of sodium dichloroisocyanurate as a disinfectant in swimming pools, drinking water and water used by food makers has contaminated water with cyanuric acid.

Figure 1: Melamine and its applications in various industries

The consumption of rice, fruits, beef, mutton, processed meats and eggs was shown to be substantially associated with the concentration of melamine in urine. A study on the link between dietary intake and melamine exposure in persons in Shanghai has found. Further findings revealed that over 85% of urine samples contained melamine, with an average value of 2.52 $\mu g\ g^{-1}$. According to research in animals, melamine is primarily excreted through the urine and renal systems and has a high rate of stomach absorption. Melamine and its metabolites (ammeline, ammelide, cyanuric acid) concentration in meat, fish and sea food, cereal products, beverages, cooking oil, and vegetables obtained in the united states indicate that meat (23.6 $ng\ g^{-1}$) and cereal products (20.9 $ng\ g^{-1}$) had significantly higher melamine and derivative concentrations than other food categories. Melamine was detected in 32.2% and 24.4% of the first and second milk samples, respectively, in research aimed at determining its presence in human milk. Meanwhile, two positive contamination samples with melamine were detected in 16.7% of women studied[3]. In September 2008, the WHO released guidelines on food-borne melamine levels, as well as reports on toxicity and preliminary risk assessment. In December 2008, the WHO held an expert meeting to study the toxicological aspects of cyanuric acid and melamine in cooperation with the food and agricultural organization, with funding from Health Canada, the maximum limit of 1 mg/kg for powdered baby formula, 2.5 mg/kg for food (apart from infant formula), and 0.15 mg/kg for melamine in liquid infant formula were eventually established in 2012 by the Codex Alimentarius Committee [CEC]. The US Food and Drug Administration, the European Community, other nations and regions established the criteria for maximum residue limits [MRLs] for melamine in response to melamine contamination occurrence. The most widely used melamine standards, set by several nations, were 1mg\kg for baby formula and 2.5 mg\kg for any other food or any other milk-based item. The Food and Safety Authority of India announced a new regulation on melamine in milk and milk products on January 5, 2016. The Food and Safety Standards Regulations, 2011 have been amended to include a new section titled 'other contamination. The allowable amounts of melamine in infant formula are 0.15 mg\kg in liquid form,1 mg\kg in powdered form, 2.5 mg\kg in other foods [4.]

2. CHEMISTRY OF MELAMINE:

Melamine is made using a proprietary method. It is predicated on distinct melamine chemistry that yields a non-thermoplastic, cross-linked polymer comprising melamine units connected by ether bonds between methylene and dimethylene. Melamine's methylol derivatives combine during the polymerization reaction to create the three-dimensional structure depicted in Figure 2. An organic base with a 1,3,5-triazine structure, melamine is a trimer of cyanamide. Similar to cyanamide, 66% of its mass is nitrogen. Melamine releases nitrogen gas when burned or charred, which causes it to lose its fire-retardant qualities when combined with resins. Since melamine is a metabolite of cyromazine, it is regarded as a pesticide. It develops in the bodies of mammals that consume cyromazine.

In 1834, the German chemist Justus von Liebig created melamine for the first time. At that point, calcium cyanamide undergoes a conversion to diacyandiamide, which is heated to its melting point to yield melamine [5].

2.1 Structure [6]:

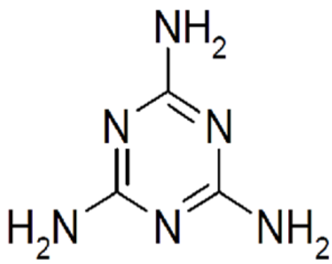

Figure 2: Structure of melamine

IUPAC names: 1,3,5- TRIAZINE-2,4,6-TRIAMINE

Other names: 2,4,6-triamino-s-triazine, Cyanurotriamide, Cyanurotriamine, Cyanuramide

Trade name: Formica and Melmac

2.2 Physio-chemical properties of melamine:

Table 1: Properties of melamine.

Classification	Melamine
Chemical formula	$C_3H_6N_6$
Appearance	White solid
Density	$1.573 g/cm^3$
Molecular weight	126.12
Molar mass	$126.123 g{\cdot}mol^{-1}$
Melting point	343°C
Boiling point	Sublimes
Vapor density	4.34
Solubility in water	3,240 mg/L at 20°C
Solubility	Very slightly soluble in hot alcohol, benzene, glycerol, pyridine, insoluble in ether, benzene, CCl_4
logP	-1.37
Acidity (P K_a)	5.0
Basicity (PK_b)	9.0
Refractive index	1.872
Magnetic susceptibility	$-61.8{\cdot}10^{-6} cm^3/mol$

3. MELAMINE CONTAMINATION IN FOOD PRODUCTS:

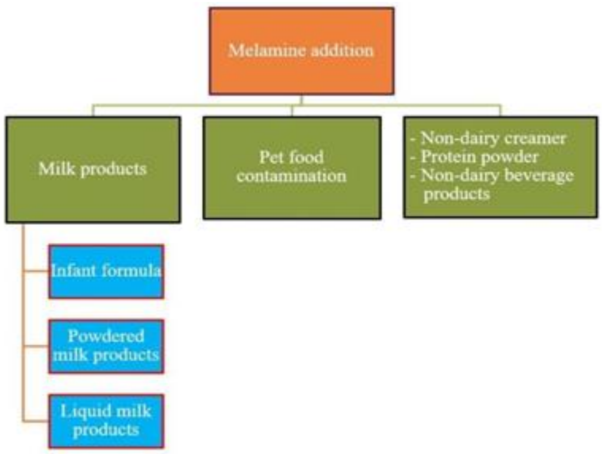

Figure 3: Flow chart showing melamine in different food products

3.1 Transmission of melamine to milk:

Melamine can enter milk and dairy products through a several different channels, including the following:

A. Artificial protein produced by adulterating milk products.

B. the use of cyromazine pesticide on crops (the pesticide is converted by the body into melamine, which can contaminate milk and tissue).

C. Applying fertilizers high in nitrogen, especially those that use melamine as a source of nitrogen.

D. Eating crops tainted with cyromazine or melamine.

E. Melamine migration from polymers used in materials for milk packaging[7].

3.1.1 Infant formula: High melamine levels in baby formulae were linked to kidney stones in formula fed newborns during the 2008 Chinese health outbreak. Over 51,900 hospital admissions, six fatalities, and an estimated 294,000 newborns in China were impacted. Three to six months after consuming the formula, symptoms appeared in 99% of the children who were impacted, all of them were younger than 3 years old, as their primary or only food sources being formula. Melamine urate crystals were found at about equimolar level in the stones that were later examined; cyanuric acid was not present. Infants excrete five to eight times more uric acid than adults do, which may act as predisposing factor for other production of melamine urate stones. The risk of stone formation may be higher in infants with inborn metabolic abnormalities, leukemia, lymphoma, or other illnesses with increased uric acid output melamine in comparison to other milk products and secondary contaminated goods, one company's line of infant milk formula products had a melamine level of up to 6,197 ppm, which was significantly higher. Comparatively, 0.137 ppm of melamine and 0.247 ppm of cyanuric acid were detected in two US infant formulas. Approximately 1/10,000 of the melamine quantities seen in many of the tainted Chinese newborn formulae, the level in the two US brands below the 1ppm current limit for infant formula. Hong Kong research conducted after the Chinese outbreak found no higher incidence of kidney stones in 3,170 patients under the age of 12 who were taking an estimated dose of 0.01-0.21 mg kg^{-1} day^{-1}.[8]

3.1.2 Powdered milk products: The China milk powder controversy involving melamine first surfaced in October 2008. To make milk appear to fulfill China's national standard for milk protein, melamine had been unlawfully added to the milk to falsely enhance its presumed protein concentration as determined by nitrogen measurement. This misleading practice affected a minimum of 294,000 children. Approximately 52,000 newborns were admitted to hospitals due to urinary stones caused by melamine, and at least 6 of them lost their lives because of this illicit activity. A Chinese company called Sanlu was the primary manufacturer for the formula milk powdered that was consumed by all affected infants with melamine- related urinary stones [MUS][9].

3.1.3 Liquid milk products: Melamine has also recently been discovered in Chinese-made liquid milk products. Mostly because protein content in China is usually measured by measuring nitrogen content, some illegal businessmen add inexpensive nitrogen-containing chemicals, like melamine, to increase protein content, which can result in cost savings for the suppliers[10].

3.1.4 Nondairy creamer: Concern over items containing the hazardous substance melamine increased when it was discovered that a variety of other milk based dairy goods and coffee creamer products imported from China were also contaminated. The coffee creamer imported from China by Yuchang F.C. was found to be melamine-contaminated, as reported by the Korean Food and Drug Administration (KFDA) reported in the article in Korea Times newspaper in 2008.

3.1.5 Protein powder: Protein powder imported from China was discovered to have 1.90 parts per million (ppm) to 5.03 parts per million (ppm) of melamine, according to a statement released by the Department of Health (DOH). Thirteen batches of protein powder were tested at random by health authorities, and six of them had melamine contamination, the food business uses protein powder as a permissible food additive. It's frequently used to create foam and strengthen cohesion. On Sunday, 40 samples of ham, vegetarian ham, fish paste, and cakes all frequently produced with the protein powder were randomly evaluated by the department's Bureau of Food and Drug Safety. According to Cheng, there was no melamine found in these completed goods [11].

3.1.6 Nondairy beverage products: In reaction to the melamine contamination issue that started with Chinese dairy goods, the U.S. Food and Drug Administration has stepped up inspections and product testing. The FDA's ongoing testing procedure has led to the discovery of melamine contamination in Blue Cat Flavor Drinks. Based on the FDA's findings, Tristar Food wholesale Co. Inc., the products distributor, stated a recall of many Blue Cat Drink flavors. The FDA suggests that food service providers and retailers take this product off the shelf and urge the public not to eat it. Consumers were notified by the FDA on September 26 that King Car food industrial Co. Ltd., a Taiwanese firm, was recalling seven Mr. brown instant coffee and milk tea items because they may have been contaminated with melamine.

3.2 Pet food contamination:

Numerous dogs in Asia experienced a renal failure outbreak in 2004 as a result of eating certain dog food. Later, melamine was identified as the outbreak's cause. Early in 2007, the Food Drug Administration (FDA) in the USA issued a recall of specific pet meals after they resulted in numerous dogs and cats getting sick or passing away from urinary crystals. Over a hundred products that might have been tainted were recalled. Imported from China, tainted vegetable protein products were marketed as 'wheat gluten' and utilized as a pet food additive. Some livestock raised food human food also ingested nontoxic levels. The feed was removed and disposed of. Melamine and cyanuric acid were found in pet food samples from this incident. An estimated 360-430 mg kg^{-1} day^{-1} of contaminated food was given to the animals, whereas some of the food tested had melamine concentrations ranging from 10 to 3,200 ppm (1ppm is equal to 1 mg/kg food source). Animals received an estimated dose of 360 to 430 mg kg^{-1}day-1 from the contaminated food; melamine levels in some tested meals range from 10 to 3,200ppm (1 ppm is equivalent to 1mg/kg food sources). Melamine and cyanuric acid were found in stones in the affected animals' distal tubes. Animal studies were conducted in several to ascertain both cyanuric acid and melamine are safe. Melamine, cyanuric acid or both were given to cats in a trail in escalating dosages. At doses of 181 mg/kg body weight for melamine and 243 mg/kg body weight for cyanuric acid, however, experienced the development of urinary stones. Two other animal studies showed that melamine and cyanuric acid when administered together at 400 $mgkg^{-1}day^{-1}$ produced renal stones, but when they were administrated separately, no stones were formed. These trials suggested that cyanuric acid and melamine alone were somewhat safe at low doses, but combined they led to the production of stones[12].
The melamine levels detected in different food products are given in Table 2[13].

Table 2: Level of melamine in food products

Product category	Contamination range [mg/kg]	No. of positive products
Powdered infant formula	0.1-2,569	22
Liquid milk and yogurt	0.6-648	52
Powdered milk products	<1-6,196	56
Nondairy creamer	1.5-6,694	2
Protein powder	3.8-8.3	2
Animal feed	3.3-21,000	7
Frozen processed foods	0.5-41	20
Whole eggs	2.9-4.7	4

3.3 Melamine in the environment:

A high standard of living in the modern world requires the everyday use of a vast number of chemicals. Pharmaceuticals, sunscreen, plant protection goods, feed and product supplements, personal hygiene items, cleaning supplies, and detergents are some examples of these chemicals. In addition, chemicals are employed as raw materials to make vehicles, green houses, technological gadgets, and other residential settings. It is utilized in paints and coatings for consumer and business goods, in foam mattresses and chairs, as a plasticizer in concrete, and in-car brake tubes and hoses. It is also present in inkjet ink, paints, sealants for plumbing, electrical, and mechanical applications, whiteboards, and shelves made of thermally fused melamine. Its main application is in the synthesis of melamine-formaldehyde resins, which are used in the production of commercial filters, laminates, coatings, adhesives, and molding compounds (used in the production of dishware and kitchenware). Melamine-formaldehyde resins are also utilized as fertilizers, coverings for seeds, and plants production goods. Another application is the application of fragrance in fabric softeners or laundry detergents using melamine-formaldehyde in shells. According to the European Food Safety Authority, melamine can be found in fertilizers and algaecides, but it can also trimerize from calcium cyanamide fertilizers [14].

4. ADVERSE HEALTH EFFECTS OF MELAMINE:

Melamine is said to be dangerous to breathe in or consume. Skin and eye irritation are caused by direct contact, and respiratory tract irritation is caused by inhalation. Clinical signs in pets with melamine-tainted food or melamine and cyanuric acid ingestion included lethargy, anorexia, rough hair, dull fur, piloerection, crimson tears, nasal discharge, oral ulcers, decreased intake of food and drink, and decreased body weight gain. The biochemical diagnostic indicated low urine pH and elevated blood urea nitrogen and creatinine levels. According to the histopathology of the kidneys, the tubules of the animals who passed away from renal failure had yellowish-brown crystals. Both in people and animals, the kidneys are the most often reported site of melamine or melamine-cyanuric acid toxicity. Internal bleeding, kidney tubule obstruction, and/or renal failure can result from the development of kidney stones or other tiny crystalluria inside the kidney tubules, a condition known as renal disease. Recent in vitro research has shown that melamine, with or without cyanuric acid, may not only be toxic to kidney tissue, but also to a wide variety of cell types in other areas of the animal body, such as the skin, spleen lymphocytes, liver, heart, stomach, and small intestine, as well as the central nervous system and male and female reproductive systems. Even after a mother ingests it, melamine can cross the placental barrier and move to the fetus in pregnancy, where it poisons the growing embryo and fetus. Triazine molecules can increase the concentration of reactive oxygen species and have the potential to cause membrane lipids to suffer peroxidative damage [15].

5. ANALYTICAL METHODS FOR DETECTION OF MELAMINE:

5.1 Ultraviolet spectroscopy

Melamine doesn't generally absorb UV light very well. Two maximal absorption peaks may be seen in the UV spectra of melamine at 210 and 236 nm. Melamine is utilized in ion-pair RPLC almost frequently, despite its reduced absorption at 236 nm. That said, research indicates that shorter wavelengths can yield higher detection sensitivity. At 210, 220, and 240 nm, we looked at three distinct wavelengths. According to the findings, 210 nm provided a suitable background noise level and the best sensitivity for melamine [16]. Many matrixes have been identified, including as soil, air, food, plant material or grains, animal feed, animal tissue, and biological fluids for use in dinnerware. While Chen has recently published a method for measuring melamine in food and feed samples using CZE-DAD, improper sample preparation interfered with the measurement. The extraction, purification, and quantitative detection of melamine by SPE and CZE-UV are all accomplished quickly, sensitively, and with a high degree of specificity, as this work explains. It was determined how well the approach performed in terms of producing precise and accurate qualitative and quantitative data within the appropriate concentration range. Several esculent matrixes, such as plant-based proteins, animal tissue, dairy products, and eggs, were tested for the presence of melamine using this technique[17].

5.2 Capillary Electrophoresis (CE)

Analytical methods such as capillary electrophoresis (CE) provide low sample and solvent consumption, quick analysis times, and excellent separation performance. These features make it a suitable alternative to the HPLC procedure. Although not as sensitive as mass spectrometry, CE is widely used for determining melamine in milk and dairy products CE is a rapidly growing separation technique in which high electric field strengths are used to separate molecules based on differences in charge, size and hydrophobicity. CE has been developed for rapid determination of MEL in recent years, employing several detection systems including UV absorbance diode array and MS Cernova's CE with UV detection technology can quickly detect MEL in milk powder samples with an LOD of 0.06l g/mL, making it more sensitive than other detection equipment. Micellar electrokinetic capillary chromatography (MECC or MEKC) separates neutral molecules by combining micelles with analytes. This approach was used to isolate melamine and its structural analogs. Vachirapatama and Maitresorasun (2013) devised an analytical method to detect melamine, ammeline, ammelide, and cyanuric acid in milk, with limits of detection of 0.10, 0.04, 0.03, and 0.08µg/mL, respectively. The separation process took 5.2 minutes and used a fused silica column coated with poly (diallyldimethylammonium chloride). Yan et al. employed the CE-DAD approach to detect MEL in dairy products, fish feed, and fish. A simple, rapid, and accurate method for determining MEL and related compounds (ammeline, ammelide, and cyanuric acid) using CZE-DAD, a capillary-zone electrophoresis method using diode array detection, has been effectively employed in egg, dairy, and pet feed. Separation was done using 40mm sodium hydrogen phosphate buffer (pH 9.0) as the running buffer. Chen and Yan devised a sensitive, easy, and reliable analytical method for determining MEL and 5-hydroxymethylfurfural (HMF) in milk. This analytical approach incorporated CE and DAD. Sodium dodecyl sulphate is utilized as a sieving matrix in the detecting system to improve separation efficiency. The approach detected MEL and HMF based on migration durations and UV absorption spectra, as demonstrated in studies. The limit of detection (LOD) for MEL and HMF assays was 0.04lg/mL and 0.067lg/mL respectively. MEL results were comparable to HPLC. CE offers advantages such as high resolution, low analysis time, and small sample sizes.

5.3 High-performance liquid chromatography (HPLC)

HPLC is a popular approach for detecting MEL, with numerous proposed methods for quantitative determination. Ehling et al. used HPLC to identify MEL, ammeline, ammelide, and CYA simultaneously in rice, wheat, and corn flours. MEL has a limit of detection (LOD) of 5ppm. Ding et al. used HPLC-DAD with a 10lg/mL limit of detection to look at MEL residue in protein powders derived from plants. Kim et al. used the same equipment to measure MEL in pet food using enzyme immunoassay with an LOD of 20 ppb and HPLC-DAD with an LOD of 0.1 g/ml. The insensitivity of HPLC-UV-based melamine detection techniques can be attributed to melamine's UV spectra, which display absorption bands below 250 nm, a region typical of many organic substances. Thus, to prevent quantification mistakes, particular attention to sample preparation and chromatographic conditions is required. HPLC was previously identified as the method for quickly determining the presence of melamine in raw milk in a national standard method published by the Chinese authorities in 2008 (AQSIQ and SAC 2008a). The standard applied to unadulterated raw milk and products derived from milk, with a detection limit of 0.05 mg/kg. Melamine is difficult to keep on conventional reversed-phase LC columns because of its high polarity. Consequently, hydrophilic interaction chromatography (HILIC) has been effectively used, particularly when melamine and its comparable chemicals are being determined simultaneously. To determine the amount of melamine in dairy products, Huang and Liu, for instance, created the HILIC HPLC-PDA method, which they reported had a 0.5 mg/kg detection limit [18].

5.4 Immunoassay (ELISA)

ELISA, immunochromatographic assay (ICA), fluorescence polarization immunoassay (FPIA), and other similar immunochemical analytical techniques are examples of immunoassays and associated procedures that are frequently used to identify different residues in food and the environment. However, no reports of ICA or FPIA for MEL have been made up to this point. As the highly selective antigen-antibody interaction is the fundamental basis of all immunoassays, the first step is to produce an antibody against the analyte. In the case of haptens, such as MEL, the primary consideration is to prepare a complete antigen by conjugating MEL to a carrier protein, such as bovine serum albumin (BSA). Since MEL has a molecular weight (MW) of 126.13, which is a little small for a hapten to stimulate an immune response, it is difficult to produce a specific antibody against them. To determine the amount of melamine in milk, Lei et al. (2011) created an indirect competitive enzyme-linked immunosorbent assay (i.e. ELISA). This assay uses the coated antigen that is prepared by combining ovalbumin with 3-(4,6-diamino-1,6-dihydro-1,3,5-triazin 2-ylthio) propanoic acid (hapten C). The recoveries achieved using the HPLC-MS method agreed with those obtained using the method, which had an LOD of 8.9 ng/ml. Instead, Zhou et al. (2012) created a monoclonal antibody (mab) and an ELISA based on the mab for the quick detection of melamine in milk powder and liquid milk, with a limit of detection (LOD) of 0.01 ng/ml. Cao et al. (2013) improved the sensitivity and specificity of ELISA for the detection of melamine in milk and milk powder by using a reasonable hapten modification and heterogeneous antibody/coating antigen combination. This resulted in a high correlation between ELISA and HPLC data (r =0.9902). Lastly, melamine was found in powdered infant formula by Tsoi and Wong (2015) using an improved microsphere-based flow cytometry immunoassay, with a limit of detection of 0.70ng/mL. For the analysis of MEL, there are presently just a few commercially accessible ELISA kits[19].

5.5 Mass Spectrometry (MS)

Melamine is usually analyzed in the positive electrospray ionization (ESI) mode, whereas its structural analogs are analyzed in the negative ESI mode. Consequently, if melamine detection and its structural analogs are required simultaneously, a polarity switch in the same experiment or two different runs are necessary (Wu and Zhang 2013). Zhu et al. (2009) developed an ultrasonic-assisted extractive electrospray ionization (EESI) approach for the measurement of melamine in raw milk and milk powder with little sample pre-processing. On the other hand, Huang et al. (2009) created a novel ambient ionization technique that coupled tandem mass spectrometry with a low-temperature plasma (LTP) probe to detect melamine in whole milk, milk powder, and other goods. With a detection limit as low as ppb and an analysis period of roughly 25 seconds for each sample, the suggested approach was incredibly sensitive and quick. Later, Huang et al. (2010a) used a portable mass spectrometer coupled to the same LTP probe to measure melamine in complex matrixes and whole milk. The method could analyze two samples per minute and had a relative standard deviation of 7.6 to 16.2% and a limit of detection of 250ng/mL in whole milk.

5.6 Liquid Chromatography Coupled to Mass Spectrometry (LC-MS)

Due to its high specificity and sensitivity, tandem mass spectrometry coupled with liquid chromatography is the preferred method for determining melamine, as evidenced by the International Reference Method (International Organization for Standardization 2010) and earlier approaches created by the Chinese authorities (AQSIQ and SAC 2008b), the FDA (Smoker and Krynitsky 2008), and Turnipseed et al. 2008. The melamine determination in soy milk, cow's milk, and infant formula powder was found to have limits of detection of 7, 5, and 10 ng/g, respectively. Tran et al. (2010) instead developed a rather straightforward sample pretreatment (extraction with a methanol-water mixture and double centrifugation to remove matrix components from the extracts), still using HPLC-MS/MS. Moreover, Chen et al. (2015) proposed a UHPLC-MS/MS method for the determination of melamine in liquid and powdered milk, reporting a limit of quantification of 0.2µg/kg for milk and 2.0µg/kg for milk powder. UHPLC has also recently been used as a separative technique coupled with mass spectrometry for melamine determination in food products. Lutter and colleagues (2011) also attempted to streamline the extraction and purification processes for the LCMS/MS melamine analysis of cow's milk and powdered newborn formula containing milk. The sample was weighed and then dissolved in acetonitrile/water (70:30, v/v). Protein precipitation was then achieved by centrifugation and subsequent dilution, all within a single vial. The authors recommended purification and concentration steps to prevent cross-contamination from the extracts coming into contact with other materials. A cellulose- binding domain-protein A (CBD-ProA) was used to immobilize antibodies to the magnetic cellulose microspheres, which were then activated using a melamine antibody conjugated through CBD-ProA and used to separate melamine from milk. Liu et al. (2016) proposed an LC-MS/MS method in the past years, which involved using immunomagnetic microspheres as a quick system for melamine enrichment in milk. Using traditional procedures, the authors generated a 50-fold concentration of melamine in 20 minutes.

5.7 Gas Chromatography Coupled to Mass Spectrometry (GC-MS)

Melamine and its related compounds exhibit poor chromatographic behavior on conventional GC columns, necessitating a derivatization step in the GC determination process. It has been observed that the chromatographic response of these compounds is enhanced by the formation of trimethylsilyl derivatives. The FDA created a GC-MS screen method based on this idea to determine melamine and its associated chemicals in various matrices, with a minimal reporting level (MLR) of 10µg/g. Instead, sample derivatization was carried out by Li et al. who created a method for the GC-MS measurement of melamine in dairy products utilizing a novel form of solid-phase microextraction (SPME) based on a zirconia hollow fiber. This allowed the extraction, concentration, and cleanup of the sample in a single step. Furthermore, sample derivatization was carried out by Miao et al. to determine the amount of melamine in milk and milk products. The samples were extracted using a mixture of diethylamine/water/acetonitrile (10:40:50, v/v/v) centrifuged and then subjected to GCMS/MS analysis. In place of this, Pan et al. used GC-MS to measure the amount of melamine in dairy products by raising the derivatization temperature to 85 °C. First, samples were extracted using acetonitrile and water, and then they were purified using graphite carbon/strong cation exchange (CARB/SCX) cartridges [20].

5.8 Fourier Transform Infrared Spectroscopy (FT-IR)

Pure melamine, gelatin, and their mixtures were scanned in diffuse reflectance mode throughout a wavelength range of 1100–2500 nm using 15 × 45 mm^2 borosilicate glass vials to get near-infrared spectra (NIRS). Every sample was subjected to duplicate scans with a resolution of 2 nm, which were then averaged into a single spectrum. Using an attenuated total reflectance FTIR spectrometer, Fourier transform infrared spectra (FT-IR) of pure melamine, gelatin, and their mixtures were obtained by depositing a small sample on the diamond crystal. By pressing on the sample with the attached arm, air was extracted. For every sample, a total of 32 scans with a resolution of 4 cm^{-1} in the 400–4000 cm^{-1} range were gathered and averaged [21].

5.9 Nuclear Magnetic Resonance (NMR)

According to the study, 1H NMR at 400 MHz can differentiate between infant formulae containing melamine and melamine-free infants, using the same set of spectra. The technique may also integrate individual lines to produce quantitative data following identification. The results of comparing NMR with SPE-LC/MS/MS for tainted Chinese infant formulae or candies were the same. Using NMR would greatly improve food safety as it is appropriate for both routine non-targeted and targeted studies of foods. The results demonstrate that NMR may be utilized for screening purposes at levels down to the lower mg/kg range; for instance, HRMAS can achieve the WHO melamine recommendations of 1 mg/kg in powdered newborn formula and 2.5 mg/kg in other foods[22].

CONCLUSION

Melamine is widely used in the production of plastics, adhesives, coatings, and flame retardants. Its low production cost and versatile properties have led to its incorporation into various products. These properties make it attractive for fraudulent adulteration of food and feed products to artificially inflate protein content which leads to significant health hazards in humans and animals when ingested, especially through food contamination. The kidneys are the most often reported site of melamine or melamine-cyanuric acid toxicity. Several analytical methods have been developed for the detection of melamine in various matrices, including food, feed, and environmental samples. In essence, this review underscores the importance of a comprehensive understanding of melamine's risks and the continuous improvement of detection techniques to mitigate its adverse effects on human health and the environment. However, despite advancements in detection technology, challenges persist, particularly in achieving high sensitivity and selectivity, especially in complex food matrices. Continued research and innovation are essential to further enhance detection capabilities and ensure the safety of food and consumer products.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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