COMMENTARY

Melamine adulteration of food: detection by point-of-care testing tool

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Melamine has emerged as one of the major food adulterants world over. Its high nitrogen content makes it a cheaper protein alternative, resulting in illegal adulteration of food and feed. Despite past incidences showing a higher risk to humans, there is no single method available to detect melamine in human samples at a lower cost-setting. Since melamine is excreted in the urine in an unchanged form, it can be detected directly by the development of a test at a patient's bedside via exploiting the intrinsic fluorescence ability of melamine cyanuric acid complex. Such a simple test will encourage huge public awareness and participation.

Melamine is recognized as one of the major food adulterants in many countries including India. Chemically melamine (also known as tripolycyanamide) is a trimer of cynamide with a 1,3,5-triazine skeleton. It exists as a white crystalline solid with partial solubility of 13.4 g/l at 50°C. Melamine is widely used in laminates, glue, dinnerware, adhesives, moulding compounds, coatings and flame retardants[1]. Due to its high nitrogen content (66%), melamine was initially used as a cheap protein alternative in cattle and pet feed, but later its deleterious effect became obvious mainly because of its slow metabolism. Recent cases of food adulteration have shown that melamine is added illegally in food items to falsely inflate their apparent protein content. These so called 'protein-rich foods' give false-positive values in standard protein assays other than the dye-binding assay. Though melamine adulteration was practised for quite a long time, it came to light in 2008, after the illness of around 3 lakh infants and death of 6 others in China, where the infant formula, milk and milk-derived products were found to be tainted with melamine. In 2007, illegal adulteration of pet food caused illness and death of dogs and cats due to kidney failure, owing to the presence of melamine–cyanuric acid crystals in the kidney of these animals. These and many other incidences have clearly demonstrated melamine toxicity and therefore, melamine is found to be a highly hazardous addition in foodstuff[2]. Even after all these mishaps, the threat of recurrence of melamine adulteration continues to loom large, especially in the developing countries (Table 1). There are many issues regarding melamine adulteration that need immediate attention.

Melamine toxicity

Melamine toxicity has been undoubtedly proved in animal models, as well as in cell lines. Melamine is known to form crystals with uric acid and other related compounds like cyanuric acid. These crystals may result in kidney damage due to obstruction of the urinary tract. Previous outbreaks have shown that melamine, when consumed in high doses for long duration, may cause nephrolithiasis, hydronephrosis, haematuria, dysuria and renal parenchyma damage both in humans as well as in animals. Melamine possesses low acute toxicity with an LD50 of 3.161 g/kg body wt in rats[3]. Direct contact as well as oral ingestion causes problems like irritation, nausea, vomiting and diarrhoea. It is also reported that chronic exposure to melamine causes reduced fertility as well as foetal toxicity. Melamine is shown to have a carcinogenic effect in rats in which it produces bladder calculi. It was found to be non-genotoxic, but was reported to cause sperm-cell abnormality. Potential for transplacental transfer and lactational transfer has been reported in animal studies. Melamine is known to generate reactive oxygen species and stimulate inflammation in cell lines such as NRK 52e, HEK 293 and RAW 264.7 by activating NADPH oxidase[4]. Numerous studies have been performed on the risk assessment of melamine in human population and they have led to the establishment of the tolerable daily intake (TDI) and maximum residue limit (MRL). TDI can be defined as the maximum amount of a substance that can be consumed daily by an individual in a population for the lifetime, without any health hazard. MRL is the maximum concentration of a residue which is acceptable in a food item. TDI for melamine, as established by WHO, is 0.2 mg/kg body wt/day, whereas MRL is different in different parts of the world; for instance, it is 0.5 mg/kg and 1.0 mg/kg for infant formula in Japan and the US respectively[5].

High risk to humans

From the animal studies of melamine toxicity and previous human outbreaks, it can be clearly predicted that humans might be at more risk than other animal[6]. Animal studies have shown that melamine is quickly absorbed in the gastrointestinal tract with a plasma half-life of 4–5 h. It is mostly excreted in the urine with little or no metabolism. Melamine when present in appropriate amount within the system, forms crystals with endogenous uric acid present in the urine, ultimately forming urinary stones. Urinary melamine–uric acid crystal formation is highly pH-dependent and is observed to form at around pH 5.5. Humans excrete higher amounts of uric acid in their urine than other mammals because of the absence of the enzyme urate oxidase. Moreover, what really adds to the risk is the lower pH of human urine. Human infants excrete more uric acid in their urine than children and adults; this group is especially more prone to urinary stone formation. This can be a dangerous situation for the infants, among whom metabolic acidosis is common. Further, the use of infant formula in China is positively influenced by lack of breastfeeding practices. There is a misconception among mothers about having insufficient breastmilk in their body, so they prefer feeding their babies with infant formula[7]. When the formula feed is adulterated with melamine, infants are

Table 1. Public news about melamine adulteration in recent years

News report	Year	Reporting agency
Melamine scandal still haunts Chinese dairy in US market[15]	2015	Want China Times
Food fraud, a global problem[16]	2015	*News24*
FDA warns against adulterated sweets and Chinese milk, ban on sale of Chinese product extended to 2015 (ref. 17)	2014	*Times of India*
Tainted milk scandal resurfaces in China[18]	2010	*BBC News*
World keeping an eye on melamine[19]	2010	Ministry for Primary Industries, New Zealand

Table 2. Issues

Several countries are yet to lift the ban on the import of Chinese food products due to the uncertainty regarding its safety.
Lack of public awareness for the abolition of melamine adulteration.
Present technology for detection of melamine is either too costly or requires high maintenance cost.
Lack of ideas that translate to the development of new technology like point-of-care testing tool.

the innocent victims of the food adulteration process. This situation is also dangerous, because of the lack of point of care test (POCT) which can detect melamine in infant food. Considering the above facts, melamine detection in foodstuff and biological samples is essential.

Current detection technology – so near yet so far

Growing awareness regarding public health and nutrition has led to significant research in the development of methods to detect melamine. There are many methods for detection of melamine such as liquid chromatography–tandem mass spectrophotometry (LC–MS/MS), gas chromatography–mass spectrometry (GC–MS), high performance liquid chromatography (HPLC), matrix-assisted laser desorption/ionization mass spectrophotometry (MALDI-TOF) and nuclear magnetic resonance (NMR). Each method has its own strengths and weaknesses. Mass spectrometry is a highly sensitive method for confirming melamine in various food samples and is rigorously improving with new labelling methods. LC–MS/MS and GC–MS have melamine detection limits of 10 and 0.002 mg/kg respectively, in milk and milk products[8,9]. MALDI-MS has a melamine detection limit of 12.5 mg/ml from urine. However, lengthy sample preparation and high establishment cost are a major setback for this method[10,11]. HPLC provides another option with a detection limit of 0.035–0.110 mg/kg in milk and dairy products[12], but at times fails to confirm the target analyte[10]. Though these techniques are highly sensitive, they are also expensive, limiting their availability in common clinical laboratories and additionally demand high expertise for operation. Enzyme linked immunosorbent assays (ELISA) is another powerful approach for melamine detection. This has advantages such as low cost, easy sample preparation and simultaneous analysis of a large number of samples. However, the tendency of the antibodies to cross-react with related compounds gives rise to false-positive results that greatly reduce the potential of this technique[11]. In spite of the presence of sensitive detection techniques, the threat of melamine adulteration still exists. The pitfall is that, so far no simple and user-friendly method has been developed for melamine detection. The present need is to develop a simple detection method with higher specificity and considerable lower limit of detection of melamine, which can be routinely used in common clinical laboratories.

Urinary melamine detection

The major route of removal of melamine after consumption with food is through the urine. Since melamine is hardly metabolized, there is a fair chance of its detection from urine directly. Therefore, we need to develop a sensitive, specific, precise and user-friendly test for the detection of melamine in the urine. Such a test, if it can be done at a patient's bedside (POCT) or used as a spot test for food items, may prove to be effective for controlling melamine food adulteration[13].

Fluorescence spectroscopy based detection – a potential approach

To develop an easy and user-friendly testing tool for melamine detection, a preliminary study was performed to analyse the intrinsic fluorescence ability of melamine alone and also in the presence of some complex forming agents (cyanuric acid and barbituric acid), when excited at various wavelengths. Melamine was analysed for fluorescence emission at acidic, neutral and basic pH at various excitation wavelengths. Further fluorescence properties of melamine–cyanuric acid complex and melamine–barbituric acid complex were analysed. No intrinsic fluorescence was observed in melamine per se, but a milky-coloured complex was formed on reaction of melamine with barbituric acid and cyanuric acid. In fact, melamine–cyanuric acid complex at neutral pH emits fluorescence at 700 nm when excited at 350 nm in a dose-dependent manner. The observed fluorescence emission property of melamine–cyanuric acid complex can be further explored for point-of-care detection of melamine from biological samples[14]. The rapid detection of melamine by POCT will be helpful in alarming the society beforehand, if melamine tainted food items are consumed. POCT tests can be effective in areas where sporadic incidences of melamine adulteration have been found to occur.

Conclusion

Increasing worldwide concerns about food safety issues demand development

of detection methods, so that food items can be routinely assessed. POCT or spot tests can be done by even minimally trained persons. This may encourage public participation which is highly desirable to control food adulteration. There are many issues concerning melamine adulteration which need immediate attention (Table 2). Today, food adulteration is a growing reality in the market, which can be prevented using simple detection measures and substantial public support. Until a POCT is developed for melamine detection, breastfeeding can prevent melamine toxicity in children[7].

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