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Overview of Melamine: Source, Contamination, Toxicology and Health Aspects

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ABSTRACT

Melamine, a trimer of cyanimide is a versatile organic compound that has several industrial applications. It contains 66% of nitrogen by mass and due to this high nitrogen content, melamine is added to different feed and food products to boosts their protein levels. It is employed in manufacture of plastic, laminates, wares, floors, table tops, paints, adhesives, molded furniture, foams, flame retardant, colorant in inks, etc. However, from last few decades it emerged as a culprit in term of adulterant in protein rich feed, food and related products and became notorious to human health. Melamine found to be one of the poisonous substances that cause deleterious effects in the organs of the human body such as renal impairment, liver function, central nervous system dysfunction, dysfunction in digestive system and reproductive systems. Due to this adulteration, many infants, across the world had died and many adults were detected with serious health issues. Melamine causes stone formation when ingested chronically in excessive doses. In the present review, we have summarized the Sources, contamination, risk assessment and toxicology of Melamine.

KEYWORDS: *Melamine; Toxicology; Adulteration; Cyanuric acid; Food supplement*

INTRODUCTION

Melamine (2,4,6-triamino-1,3,5-triazine, $C_3H_6N_6$) a white organic base, nitrogen rich crystal compound primarily used in the synthesis of melamine formaldehyde resins for the production of paper finishers, commercial filters, molding compounds, wrinkle-free textile, dishware and kitchenware and many other materials [1].

As such, Melamine is not a naturally available and is not approved for direct addition to food products however; it is approved for use as part of certain food contact substances. 2008 mark the year when an increased in incidence of kidney stones and renal failure among infants has been reported extensively in China. And the culprit found was melamine which was deliberately added to infant formula. Previous outbreaks of renal failure related to melamine have been reported in pets

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in 2004 in the Republic of Korea and in 2007 in the United States when the substance was added deliberately to a pet food ingredient [2].

Predominantly, melanin is added to increase the nitrogen level and to reduce the cost of the several food and food related products like, dairy products, coffee drinks, infant formula and pet food. In spite of the fact that melamine alone has less toxicity, but when it is combined with cyanuric acid and uric acid, which can form insoluble crystals that leads to kidney stones, renal failure and in severe cases ultimate death [3]. Commercially, melamine is synthesized from urea with production of cyanic acid at intermediate step and other byproducts such as cyanuric acid, ammeline and ammelide. Melamine is converted into cyanuric acid by hydrolysis process and reverse in melamine form by amination process **[4]**. The molecular weight of melamine is 126.12 g/mol containing Nitrogen 67% by mass. In industry, melamine resins are produced by combining melamine with formaldehyde, which is a very durable thermosetting plastic, polymer cleanser and melamine foam. Melamine is also found in various products like dry erase board, counter-drops, glues, fabrics, textile, kitchenware and flame resistors product. In pigment yellow-150, melamine plays a major role, which is used as colorant in inks and plastics. Moreover, it is a derivative of arsenical drugs and melarsoprol.

Toxicology and risk assessment

Melamine and its chemical analog cyanuric acid have rapid absorption and excretion and almost completely unmetabolized in the urine. Both these compounds have elimination half-lives about 3hrs [5].

Toxicity of melamine

Many reports proved that the toxic substance melamine can cause health hazardous problem in human body, such as change in anatomical, cellular and functional aspects in different organ of human body such as in central nervous system, cardio vascular system, liver, kidney, reproductive functions and many others. Some cellular changes such as nephrolithiasis, hydronephrosis, urolithiasis occurs due to the melamine. Reported toxicity of Melamine was shown in figure1.

Melamine has been reported to be being harmful if swallowed, inhaled or absorbed through the skin. In the year 2007, United States reported the thousands of sudden death cases of domestic cats and dogs, later the scientist discovered the culprit for this tragedy was China imported wheat gluten, which is important source of plant protein. After one year one more tragedy was reported in China exported baby formula consisting of inedible melamine due to problematic gluten. These Chinese products were also exported to Hong Kong, Macau, Taiwan, Singapore, Japan, South Korea, and Australia. In all these countries the infants and young children was suffering with stones, and melamine crystals in kidney, urinary bladder and urine, hematuria and hydronephrosis [6,7]. In China, more than 300,000 babies were suffered and 6 babies were died in melamine incident.

In rats, oral dose of melamine shoes low acute toxicity which exceeds above 3.161g/kg of body weight where as in acute dermal toxicity is seen when exposer is 1g/kg of body weight [8,9].Oral consumption of melamine shows the harmful effect on GIT tract including symptoms like nausea, vomiting and diarrhea. Along with this direct contact can cause irritation of skin and eyes and inhalation causes infection of respiratory tract. Animal study reported that long term exposure to melamine reduces the fertility which is responsible for the fetal toxicity. The most common problem reported is chronic renal toxicity i.e. stone formation [10].

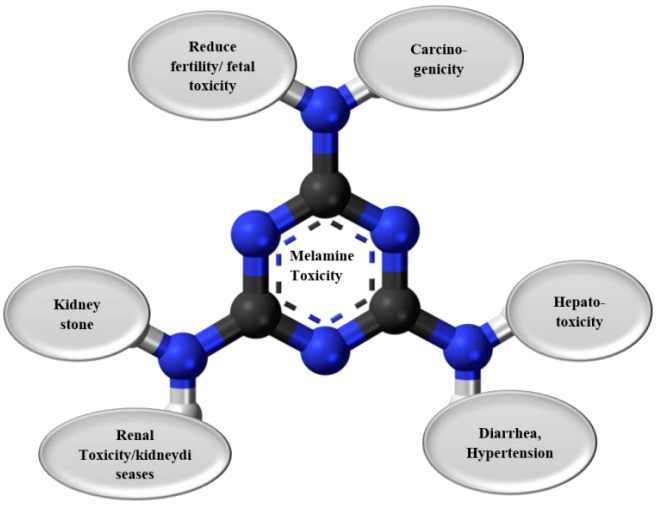

Figure 1: Melamine toxicity.

Melamine has low acute toxicity in mice and rats when lethal dose is given orally that exceed 3,000mg/kg body weight. The excretory organs, kidney and bladder are mostly affected by the toxicity of melamine. Bladder stone formation and hyperplasia of the bladder epithelium were significantly included in the dose related effects. The males are more susceptible to these effects as compared to females [11].

Studies of the melamine toxicity with human exposure are limited, and all this available toxicokinetic and toxicological data has been collected from the animal studies. As melamine and cyanuric acid have high range of lethal doses (LD50's) they are not estimated as acutely toxic. LD50's range of melamine from 3.1 g/kg to more than 6.4 g/kg in rats and 3.2 g/kg to 7.0 g/kg in mice. In pig melamine shows low volume of distribution 0.61 L/kg and a half-life of 4hrs and little metabolism with substantial renal excretion of unchanged [12,13]. Elimination of the cyanuric acid is also rapid.

In 1953, Hazleton Laboratories performed the experiment on dogs by giving them food containing 3% (30,000 ppm) melamine by weight to study the chronic urinary changes. Distinctive urinary changes, with reduced specific gravity, increase in urine rate,melamine crystalluria, and protein in urea with microscopic hematuria was reported from this experiment [14,15].

In 1983, NTP studied the carcinogenicity of melamine by oral ingestion in rats and mice. The consequences found from this study were urinary bladder carcinoma in male rats, whereas male mice observed with urinary bladder hyperplasia. In female rats, chronic inflammation of the kidney and chronic nephropathy in aged rats was noticed by NTA [11,16].

Earlier observation has proved that (95% to 99.9% pure) melamine is capable of forming cancer in high doses when added to food products. All these tumor findings are firmly related with the calculus formation and exposure to high doses.

In year 2004 and 2007, two tragedies of acute renal failure were reported in cats and dogs in South Korea and United States respectively, and the responsible factor was food products adulterated with the melamine and cyanuric acid [17]. As per the studies in humans and animals, melamine toxicity is the result of the crystal deposition in the distal tubular lumen, which leads to necrosis, corticomedullary hemorrhage, and inflammation [18,19].

Various studies on rats have reported that (95% to 99.9% pure) melamine is cancer forming in high doses when added to their feed. This cancer in significantly affects the bladder of animals which leads to stone formation. It has suggested that tumors not only grow because of genotoxic effect of chemical only but also the result of bladder epithelial irritation and hyperplasia [20, 21].

Toxicity of cyanuric acid

The melamine and cyanuric acid have somewhat similar toxicological profile. At higher doses low acute toxicity and sub-chronic toxicity were reported in low incidence of bladder calculi at concentrations up to 5,375 mg/L in drinking water.

Combined toxicity

From the earlier experimental studies and the tragedies in pets and livestock, investigating the combined effect of melamine with cyanuric acid in cats [22] and in fish and pigs [23], oral consumption of melamine with cyanuric acid causes more severe renal damage than exposer individually.

The urine of the infected cats when tested showed the amorphous and fan shaped birefringent crystals. Severe renal interstitial edema and hemorrhage at the corticomedullary junction has been found in the cross section of kidneys. Histopathologic findings were limited to the kidneys, and numerous crystals were found within the distal nephrons associated with tubular epithelial necrosis and regeneration. In case of the chronic infection, lymphoplasmacytic or granulomatous tubulointerstitial inflammation and fibrosis were observed with larger crystals [17,24].

Consistent renal stones were reported when only melamine is administered orally, in centimeters and melamine with cyanuric acid leads to the formation of crystals measuring between 20 µm to 200 µm causes rapid renal failure having similar mechanism to acute uric acid nephropathy, not reported with ingestion of melamine alone.

Risk Assessment

The subchronic studies in rats is the basis of the risk assessment and most significant result is bladder calculi formation which is dose dependent or local concentration dependent that do not show any sign of accumulation. A 95 % lower bound of the 10 % benchmark dose calculated from the sub-chronic feeding studies of male rats combined data with the help of dose response modelling. Dietary conversion and an additional feed intake reduction factor of 14% led to the BMDL10 of 35 mg/kg body weight per day. Tolerable daily intake (TDI) of 0.2 mg/kg was observed when uncertainty factor was 200. The uncertainty factor consists of the default 100-fold factor with extra 2-fold factor to detect the sensitivity of infants and also for data uncertainty in relation to possible under reporting of bladder calculi because of preparation of tissues [11].

For adults estimated exposure level of melamine from “baseline” i.e. level in food that does not result from all sources, was estimated up to 13 µg/kg body weight per day. From this incident conservative estimates from adulterated products were 0.8 to 3.5 times the TDI. For comparison, estimated exposure of infants in China to adulterated infant formula, at median levels of the most affected brand, ranged from 8.6 mg/kg to 23.4 mg/kg body weight per day. These levels are about 40-120 times the TDI and explain the dramatic health outcome in Chinese infants [11].

Sources and levels of contamination

Urea is used as raw materials in the production of melamine [40]. Food items such as, Beverages (coffee/orange juice), powdered milk formula, liquid milk, contaminated foods, processed foods and ingredients, whole egg found to contaminated with melamine [25]. The various sources of melamine are depicted in (Figure 2).

Food products can be contaminated through the melamine intentionally and unintentionally to increase the false protein in nutritional substance [26]. Melamine is mainly used in the manufacture of tableware, cans, plastic package, cleansing foam etc. It is also used in plasticizer as well as in fertilizer.

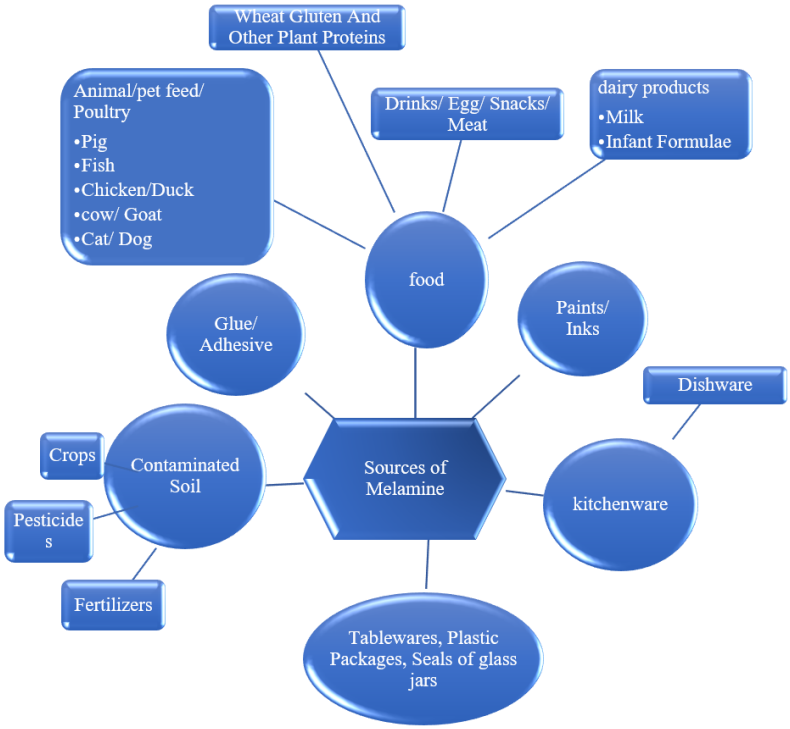

Figure 2: Sources of Melamine.

Intentional Contamination

In 2004, Melamine first found contemplation as far back in domestically pet feed issues [26]. In Asia a tragedy of renal failure was found in many dogs followed by the consumption of contaminated food products and source of outbreak melamine was implicated later. The USA Food and drug Administration (FDA) call back certain domestic pet foods developing urinary crystals due to which many dogs and cat become ill or die, and also recalled more than 100 potentially contaminated products [27,28].

In some tested food amount of melamine found in range from 10 to 3,200 ppm (1 mg/kg food is equivalent to 1ppm) in animal found 360 to 430 mg per kg approximately dose in day 1 from tainted food. The melamine and cyanuric acid were present in the tissue of animal fed and in affected animal stones in distal tubules also contained melamine and cyanuric acid and it's identified after histological investigation [22].

In the 2007 tragedy, detected in adulterated pig feed very high levels of ammelide, ammeline, melamine and cyanuric acid ranging from 9,200-29,000 mg/kg (melamine), 39,000-92,000 mg/kg (ammelide), 20,000-34,000 mg/kg (ammeline) and 2200-9100mg/kg (cyanuric acid) found in that feed [29]. This notorious contamination further in China 2008 disturbed the entire cosmos by reaching baby formula. It spread to snacks like biscuits, ice-cream and chocolate because their ingredients were contaminated. Unfortunately, it wasn't limited to consumption of infants. Melamine was also found in eggs, wheat gluten, and other food stuff. Several brands in United states like biscuits, candies, coffee, milk, chocolates and other drinks which is made from china ingredients withdrawn from the market because of contamination [19].

A diary company namely Sanlu group sealed more than 2000 tons of tainted milk powder and recollected about 9000 tons from the market [30]. In 2010 regrettably, more than 170 tons returned to the market and once again was recalled [31]. Later, more news exposed that some of the tainted product was sold to farm to feed the piglets [32]. Twenty-two companies were detected with melamine in 69 batches [33]. In infants several reasons for melamine increased risk of stone formations like errors of metabolism, leukaemia, lymphoma, or other illness that can increase uric acid secretion. In one company's in infant milk formula products reported melamine level up to 6,197ppm, which was much higher level found than other tainted milk products and contaminated secondarily foods [34].

Unintentional contamination

This chemical is around us in our daily life, for example, plastic packages, paints, tableware, glues, cleansing foam, cans and seals of glass jars and adhesives even there is no purpose of addition melamine to foodstuff [35,36].The melamine product popular as a melaware or melamine-ware because of its shiny outlook that agglomerate ceramic but it has non-breakable property. In microwave ovens some melamine ware can withstand up to 140°C.Melamine formaldehyde resin is able to evacuate from tableware to beverages or food [37,38].

As melamine is economical and easily available industrial material, so for increasing the nitrogen level and making it affordable was added to various dairy products, infant formula, biscuits, candy, coffee drinks, pet food and feed. Milk contains higher melamine followed by Yoghurt, Cheese and infant formula [39].

Melamine was reported in pesticide cyromazine in plants, goats, hens and rats (JMPR Report 2006) [40]. As it contains nitrogen and it is used in nitrogenous fertilizers for growth of food crops. Melamine leaching occur in food equipment, containers or packaging materials that comes in contact with food materials at high temperature, but that does not have any serious effect to human health [41]. Apart from low level of residue as result of cyromazine metabolism it might be occur through the acidic foods like lemon, orange juice and curdled milk at high temperature, which extracts the melamine from compression moulds. This shows the oral intake of melamine is approximately 0.007 mg/kg body weight per day [40].

The Tolerable daily intake (TDI) of melamine and its structural analogues (ammeline, ammelide and cyanuric acid) as per United States food and drug Authority (FDA) is 0.63mg/kg body weight. The US-FDA suggested that in any food melamine and its derivatives shouldn't found more than 2.5 ppm within above TDI. In infant formula melamine should not contain more than 1 ppm and other foods not more than 2.5 ppm [41,42].

FSSAI permissible limit

The FSSAI has added new regulation on through notified by 5th January, 2016 for limit of melamine in milk and milk products. In a new section according to amendment it has added to regulation called “Other contaminant” in the Food Safety and Standards (Contaminants, toxins and Residues) Regulations, 2011.The melamine maximum limits content are 1mg/kg. [43].

CONCLUSION

Melamine basically an industrial product added to animal feed and infant formula purportedly to increase nitrogen level and protein content and has been detected in many milk and milk-containing, food and feed products; Melamine causes stone formation when ingested chronically in excessive doses. The normal tolerable range of melamine for the human body is 0.2 mg/kg body weight and when this range exceeds, it shows the toxicological effects in the body. Regulatory agencies and FDA have given advisories of the acceptable levels of melamine in foods.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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