

Melamine Toxicity and the Kidney

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ABSTRACT

The toxicity of melamine caught the attention of physicians as a result of a recent spate of renal injury after exposure to melamine-tainted milk in China. Melamine is an organic nitrogenous compound used in the production of plastics, dyes, fertilizers, and fabrics. In the current incident, melamine was added to milk to elevate falsely assay results for protein content. A variety of toxic effects from melamine, including nephrolithiasis, chronic kidney inflammation, and bladder carcinoma, all have been studied in animals. We review here the epidemiology, clinical features, and investigative findings concerning the only outbreak of melamine poisoning in humans. We also examine the renal toxicities of melamine and cyanuric acid—a by-product of its synthesis—and the associated risk factors on exposure and provide guidance on levels in foods.

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Melamine was an unknown substance to nephrologists until today. Although studied as diuretics 50 yr ago,¹ it never came into clinical practice with the development of other potent and safer means for naturesis. This compound became headline news recently after the occurrence of an outbreak of urinary stones in infants and children consuming melamine-tainted milk in China. The nephrotoxic effects of melamine now warrant the attention of nephrologists, pediatricians, urologists, and radiologists.

WHAT IS MELAMINE?

Melamine is an organic base commercially synthesized from urea with an intermediate step producing cyanic acid (Table 1). The reaction also results in the formation of other byproducts, including cyanuric acid, ammeline, and ammelide (Figure 1). Melamine is 66% nitrogen by molecular weight. It is combined with formaldehyde by industry to produce melamine resin, a very durable thermosetting plastic, and

melamine foam, a polymeric cleanser. Other commercial products containing melamine include countertops, dry erase boards, fabrics, glues, housewares, and flame retardants. Melamine is also one of the major components in pigment yellow 150, which is a colorant for inks and plastics. It is also a derivative of arsenical drugs, and Melarsoprol is one such drug used for the treatment of African trypanosomiasis. Beginning in 1958, melamine has been used in fertilizers and is occasionally offered as a nonprotein nitrogenous source for feeding cattle. Subsequently, however, it was shown to be an ineffective nonprotein nitrogen source for animals because of its slow hydrolysis in ruminants (Table 1).

WHY DID MELAMINE APPEAR IN MILK?

Melamine was recently added to milk to elevate falsely its protein content by the Kjeldahl method (Figure 2). The first step of the Kjeldahl method detects not only nitrogen in protein but also nitro-

gen in all organic nitrogenous compounds, including melamine. In essence, nitrogen in the assay reacts with sulfuric acid to form ammonium sulfate. Small amounts of sodium hydroxide are then added, and the ammonium salt is converted back to ammonia. Ammonia is reacted with sulfuric acid again, and the remaining acid is quantified by adding sodium carbonate with a methyl orange pH indicator to quantify the amount of ammonium salt (Figure 2).

The addition of 1 g of melamine to 1 L of milk falsely increases the protein content by 0.4%. When melamine is dissolved at the room temperature, 3.1 g of melamine can be dissolved in water without forming precipitate, and protein content will falsely increase by 1.2%. This can roughly lead to an overestimation of the protein content in liquid milk by 30%. In case of milk powder, the amount of melamine added can be greater because of its greater solubility at higher temperature when adding warm water.

METABOLISM IN ANIMALS

Melamine is not metabolized by animals and is rapidly eliminated in the urine. More than 90% of ingested melamine is

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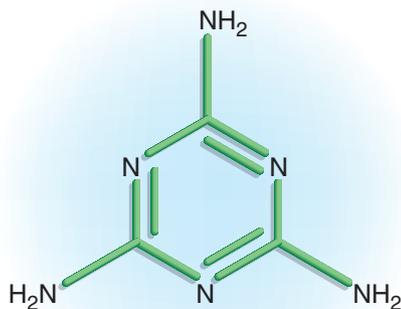


Figure 1. Chemical structure of melamine: There are six atoms of nitrogen in a molecule of melamine, constituting 66% of molecular weight. International Union of Pure and Applied Chemistry name: 1,3,5-triazine-2,4,6-triamine; other names: cyanurotriamide, cyanurotriamine, or cyanuramide.

excreted within 24 h. The half-life of melamine excretion in animal studies ranged from 2.7 to 4.04 h.^{2,3} The levels of melamine in blood, liver, or plasma are similar.² The volume of distribution of melamine in pigs is 0.61 ± 0.04 L/kg and is not extensively distributed to most organ tissues.³ This study in pigs also confirms the excretion of melamine best fits a one-compartment model in which melamine has a half-life of 4.04 ± 0.37 h and a renal clearance of 0.11 ± 0.01 L/h per kg (approximately 27 ml/min).³

TOXICITY OF MELAMINE

Studies concerning the toxicity of melamine taken orally in humans are nonexistent. Toxicity data mainly come from studies in sheep, cat, dog, mice, and rat.

Table 1. Summary of the chemical properties of Melamine²

Property	Description
Appearance at room temperature	Whitish crystalline solid
Solubility	Partially soluble in water 3.1 g/L in water at 20°C 13.4 g/L in water at 50°C 25 g/L in water at the boiling point
Melting point	Insoluble in diethyl ether 357°C
Specific gravity	1.573 g/cm ³
Molecular weight	126.12 g/mol

Toxicity can be classified as acute or chronic. The most common toxicity is renal toxicity, which is also the area of most concern to nephrologists.

Acute Toxicity in Animals

Melamine has a low acute toxicity; the LD₅₀, the lethal dose of a compound that would result in death in 50% of the tested animals, for melamine in rats is 3.161 g/kg body wt.⁴ Acute dermal toxicity in rabbits presents when the exposure is >1 g/kg body wt.⁵

Direct contact results in skin irritation and eye irritation, and inhalation causes respiratory tract irritation. Oral ingestion affects the digestive tract, presenting as nausea, vomiting, and diarrhea.⁶

Acute renal toxicity is best illustrated by a sheep study done in 1953.⁷ When sheep were fed a single 100-g oral dose of melamine, all of them died by the 11th day. When a daily dose of 25 to 50 g of melamine was given for 7 to 9 d, again all of the sheep died. They experienced acute renal failure with elevation in blood urea nitrogen and creatinine followed by oliguria preceding death. Postmortem examinations revealed crystals in the kidney tubules, nephrosis, and hemorrhagic cystitis. When the exposure was reduced to 10 g/d melamine for 16 to 31 d, two thirds of the sheep died. Again, they experienced loss of appetite, oliguria, and elevation of blood urea nitrogen and creatinine before their death. Postmortem analyses under these conditions also revealed crystal deposition in the kidney.

Chronic Toxicity in Animals

Long-term exposure to melamine reduces fertility and results in fetal toxicity in animal studies. The classical descrip-

tion of urinary changes relating to chronic exposure to melamine dates back to 1953, when Hazleton Laboratories performed an experiment in dogs by feeding them 3% (30,000 ppm) melamine by weight in food for 1 yr.⁸ Distinctive urinary changes were noted, including reduction in specific gravity, increases in urine output, melamine crystalluria, and proteinuria with microscopic hematuria. The most commonly reported chronic renal toxicity is stone formation. Uncertainty exists as to whether melamine results in any chronic damage to kidney other than aggressive stone formation.

Urinary Stone Formation

Most animal studies concerning subacute or chronic melamine exposure reveal stone formation. Incidence ranges from 5 to 100% depending on the dosage of melamine, gender, and amount of water intake.^{4,9} The composition of stones is either a combination of melamine and uric acid or melamine in a matrix of protein, uric acid, and phosphate.¹⁰

The incidence of stone formation increases with daily exposure to melamine. The lowest possible daily dosage of melamine that results in bladder stone formation is as low as 750 ppm for 13 wk.⁴ The dose-response curve for the induction of urolithiasis in weanling rats is extremely steep (Figure 3). This suggests formation of calculi occurs in supersaturated urine but not in urine that is undersaturated.¹¹ A study performed on B6C3F1 mice with exposure to 13 wk of melamine showed that male mice are much more affected than females despite similar body weights. Relative risk of stone formation in males is twice as great as in females.⁴

A study of exposure to melamine for 36 wk in rats demonstrated the incidence of stone formation was reduced by increasing the amount of fluid intake.⁹ In this study, one group of rats, the control group, was fed diets containing 1% melamine. The other two groups of rats received feed containing 1% melamine together with 5 or 10% of sodium chloride, respectively. The increase in salt content in the diet resulted in doubling and tri-

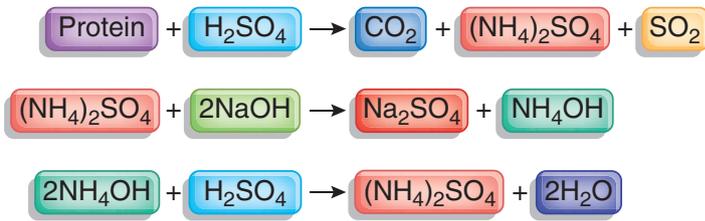


Figure 2. Kjeldahl method, a universally used quantitative method for assessing protein content in food.

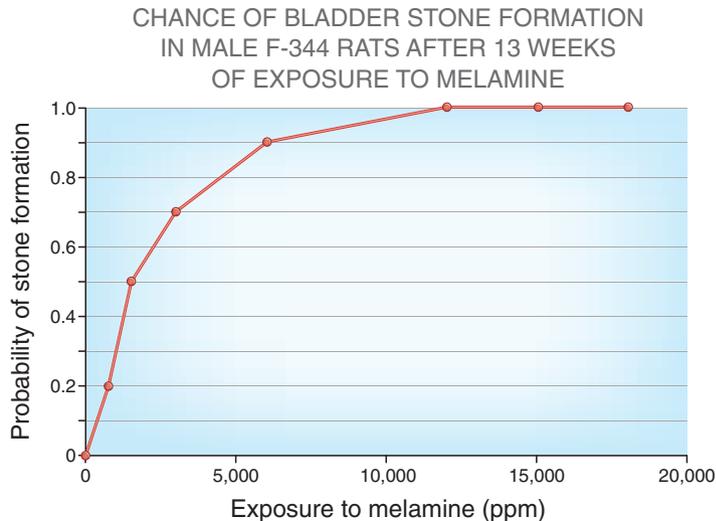


Figure 3. Chance of bladder stone formation in male F-344 rats after 13 wk of exposure to melamine. Figure drawn using data from Melnick *et al.*⁴

pling the amount of fluid intake in groups taking 5 and 10% sodium chloride when compared with control. Incidence of stone formation was reduced from 37% in control group to 11% in the group taking 5% sodium chloride and to 5% in the group taking 10% sodium chloride.

THE HUMAN OUTBREAK

The toxicity of melamine came to our attention in Hong Kong because of a recent outbreak of urinary stones in children who consumed melamine-tainted milk in China. On September 11, 2008, Xinhua News Agency in China reported an outbreak of urinary stones in children younger than 3 yr.^{12,13} The outbreak was initially linked to consumption of melamine-tainted milk powder from the Sanlu Group, a leading dairy producer in China. All of the children affected had

an exposure to the tainted milk for approximately 3 to 6 mo before the onset of stones. Later, the contamination was found in other brands of dairy product. The highest content of melamine was in Sanlu milk powder, up to 2.563 g/kg powder, whereas melamine in the other brands ranged from 0.090 to 619 mg/kg.¹⁴

The official data released by the Ministry of Health of the People's Republic of China on September 21, 2008, stated that a total of 52,857 children had received treatment for melamine-tainted milk¹³; 99.2% of the children were younger than 3 yr, although more children who were older than 3 yr were reported afterward. Some of the children were asymptomatic; however, most symptoms included irritability, dysuria, difficulty in urination, renal colic, hematuria, or stone passage.¹⁵ Hypertension, edema, or oliguria also occurred in more severe cases. Urinalyses of exposed children revealed mi-

croscopic hematuria with or without proteinuria. Melamine can be detected in the urine of the affected children by biochemical test or the presence of fan-shaped crystals.

Analyses of stone composition mainly demonstrated melamine and uric acid.^{15,16} Stones were radiolucent, and plain x-ray films failed to show their presence. Ultrasonography or computed tomography scan can reveal the presence of stones and detect whether there is any obstruction. In view of the large radiation dose of computed tomography scans for children, ultrasonography is the preferred first-line investigation.

Melamine stones in humans are characteristic. They usually occur bilaterally, and multiple stones are often present.¹⁵ The acoustic shadow of stone can be absent. They are soft in nature and can be broken up easily. Most of the stones are usually <1 cm in diameter. Stones that are <4 mm in diameter can spontaneously pass to the bladder with adequate hydration. When stones are >4 mm or show evidence of obstruction, medical treatment with hydration and follow-up ultrasonography is the first-line attempt at treatment. If conservative medical therapy fails, surgical drainage and removal will be needed. Acute renal failure occurred in 2.5% of the cases.¹⁶ Mortality was recorded in four cases.¹⁷

RENAL PARENCHYMAL DAMAGE

Uncertainty exists as to whether melamine causes direct renal toxicity on long-term exposure; however, in studies in which no chronic toxicity was observed, the dosage of melamine was usually lower.¹⁸ Two studies specifically investigated the toxicity of melamine by looking at postmortem sections of kidneys in animals. The first study from Melnick *et al.*⁴ observed a significant increase in chronic inflammation in the kidneys of female rats exposed to melamine for 103 wk compared with controls (82% in rats fed a diet containing melamine at 9000 ppm *versus* 34% in those on diets containing melamine at 4500 ppm *versus* 8% in control; $P \leq$

0.01). The observed chronic inflammation in this study could not be attributed to stone formation, because no stones were detected in the urinary tract. The male rats showed a slightly increased incidence of chronic inflammation, but this was not statistically insignificant.

The second study from Ogasawara *et al.*⁹ demonstrated ischemic changes in the renal cortex of rats (focal lesion of fibrosis, inflammatory cell infiltration, and renal tubular regeneration) after feeding on melamine for 36 wk. Ischemic changes occurred in 100% of rats fed diets containing 3% melamine by food weight and occurred in only 5% of rats when fed diets containing 1% melamine.

Melamine Toxicity Combined with Cyanuric Acid

A widely known epidemic of melamine poisoning is the infamous “pet food–induced nephrotoxicity in North America” in 2007.¹⁹ In March 2007, numerous cases of acute renal failure in dogs and cats were associated with the ingestion of a variety of dog and cat pet foods. One of the contaminants was melamine, which was added for the same reason as in the recent milk exposure in humans: To give a falsely high protein content. In this pet outbreak, not only was melamine present, but also another toxic compound—cyanuric acid—was a contaminant, giving rise to a very high mortality in these animals.

Cyanuric acid (*s*-triazine-2,4,6-triol) is structurally related to melamine (Figure 4A). It is used as a stabilizer in outdoor swimming pools and hot tubs to minimize the decomposition of hypochlorous acid by light.²⁰ How cyanuric acid got into the pet food is unknown. It could have been added intentionally or remained as a contaminant during melamine synthesis, because cyanuric acid is a byproduct.

Studies on the toxicity of cyanuric acid are limited, but it is likely to behave as melamine as a result of its structural similarity. The subacute feeding of sodium cyanurate at 700 or 2200 mg/kg in rats resulted in bladder calculi and some associated bladder epithelial changes, re-

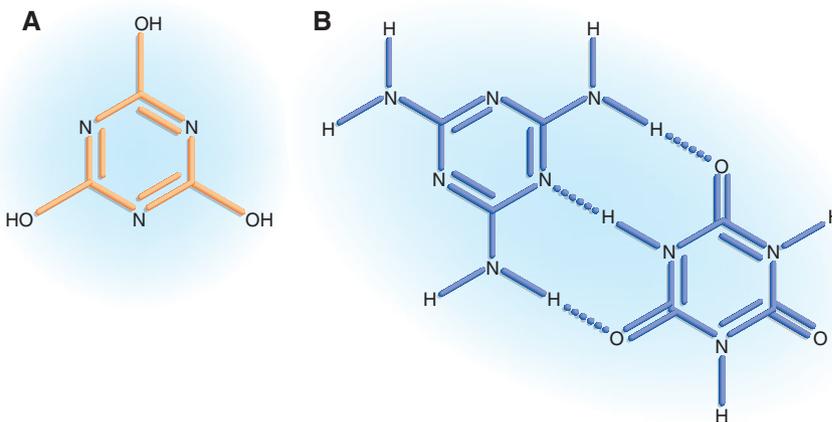


Figure 4. (A) Molecular structure of cyanuric acid, noted for its structural similarity with melamine. (B) Melamine can interact with the isomeric form of cyanuric acid to form melamine cyanurate, explaining the increase in stone formation and toxicity.

spectively.²⁰ No other adverse effects were noted.

Melamine, however, can interact with the isomeric form of cyanuric acid to create crystals (Figure 4B). Melamine combined with cyanuric acid results in acute renal failure in cats within 48 h after ingestion. The toxic dosage in their diet is as low as 0.2% melamine and 0.2% cyanuric acid in contrast to no evidence of renal failure in groups taking either melamine or cyanuric acid alone up to 1% in their diet for 10 d.²¹ Urine analyses from affected cats revealed the presence of amorphous or, in some cases, fan-shaped (Figure 5A) birefringent crystals. The crystals were a combination of melamine and cyanuric acid.²² Cross-sections of the kidney demonstrated severe renal interstitial edema and hemorrhage at the corticomedullary junction. Histopathologic findings were limited to the kidneys, and numerous crystals were found within the distal nephrons associated with tubular epithelial necrosis and regeneration (Figure 5B).²² In chronic cases, lymphoplasmacytic or granulomatous tubulointerstitial inflammation and fibrosis were found and the associated crystals were larger.^{23,24} This toxicity was not limited to cats. Similar nephrotoxicity was observed when a combination of melamine and cyanuric acid was given to fish and pigs.²⁵ The toxicity is size dependent, with cats affected more than dogs and small dogs affected more than large dogs.^{23,25} The mortality of combined

melamine and cyanuric acid is as high as 74% in dogs and 61% in cats.¹⁹

CARCINOGENICITY OF MELAMINE

There are no data concerning the carcinogenicity of melamine in humans. The carcinogenicity in animals was determined from studies in rats and mice. Exposure produces urinary bladder and ureteral transitional cell carcinomas in male rats but only urinary bladder hyperplasia in male mice. Female rats or mice did not have carcinoma, but transitional cell papillomas were found in female rats.⁴ The occurrence of urinary bladder tumors in male rats correlates well with stone formation and exposure to high dosages. A similar dosage-dependent relationship was confirmed in another study using male rats.⁹ The administration of sodium chloride to increase fluid intake and urinary output reduces the prevalence of stone and tumor occurrence.

There is no evidence that melamine undergoes biotransformation. Mutagenesis of melamine was not observed in studies of exposure to *Salmonella typhimurium* and *Drosophila melanogaster*.^{26,27} The urinary bladder tumors seen in male rats exposed to high dosages of melamine seem to be produced by a non-DNA-reactive mechanism involving epithelial hyperplasia secondary to

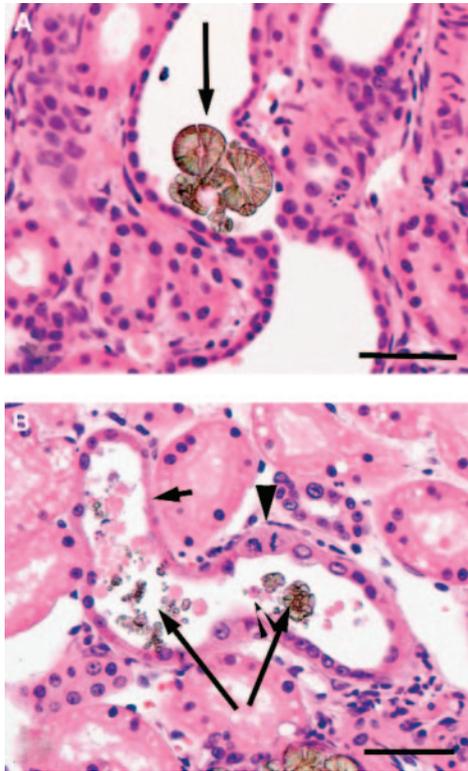


Figure 5. Histologic diagnosis of melamine-associated renal failure based on renal crystal characteristics. (A) Dilated distal cat tubule contains clusters of round green melamine/cyanuric acid crystals with radiating spokes and concentric striations (arrow). Surrounding proximal tubules appear unaffected (hematoxylin and eosin; bar = 45 μm). (B) Dilated distal cat tubule contains fragmented or globular dense green melamine/cyanuric acid crystals (long arrows). Note attenuation of the lining epithelium with wide separation of nuclei (short arrow) and mitotic figure (arrowhead) indicative of tubular epithelial necrosis and regeneration (hematoxylin and eosin; bar = 45 μm). Reprinted from Brown *et al.*,²² with permission from the American Association of Veterinary Laboratory Diagnosticians.

the presence of melamine-containing bladder stones. These studies concluded that bladder tumors would not occur in rodents unless exposed to dosages that result in bladder stones.

Melamine has been classified as a group 3 carcinogenic risk by World Health Organization,²⁸ meaning that melamine is not classifiable as to its carcinogenicity in humans, referring to the fact that the evidence of carcinogenicity is inadequate in humans and inadequate or limited in experimental animals.

GUIDANCE ON LEVELS IN FOODS

Recommendations on the safety limits of melamine in food come from animal studies. The no observed effect level

(NOEL) of melamine in studies in rodents is 63 mg/kg body wt per d. When this dosage is converted to a human safety limit, it is referred to as tolerable daily intake (TDI). TDI is usually defined as 1% of NOEL in view of the need for a tighter safety limit in humans; therefore, the TDI in humans may be equivalent to 0.63 mg/kg body wt per d, and this is why the recommendation from the Food and Drug Administration gives a safety limit of exposure to melamine and its structural analogues to be <0.63 mg/kg per d.²⁹ The European Food Safety Authority, however, recommends a daily exposure of melamine and its structural analogues be <0.5 mg/kg per d.³⁰ After reviewing the cases in China, children who are younger than 3 yr seem more susceptible to the toxic effects of mel-

amine. An additional safety factor was thus introduced to protect this most vulnerable group; that is, children who are younger than 3 yr. Consequently, the latest TDI was further reduced to 0.32 mg/kg per d.³¹ Of course, in determining exposure, one has to take into account the average body weight of the target population and the amount of respective food eaten per day to calculate the tolerable exposure of melamine per person each day.

CONCLUSIONS

With the best available evidence in human exposures and animal studies, we conclude several points regarding the toxicity of melamine: High-dosage melamine will result in urinary stones, crystalluria, and acute renal failure in both humans and animals; stone formation is likely enhanced by smaller body size, higher dosage of melamine, and smaller amounts of fluid intake; studies in animals show that males are more affected than females; toxicity of melamine is further aggravated by the presence of other impurities associated with melamine synthesis, particularly cyanuric acid; tubular damage with obstruction from crystals and chronic inflammation of kidney can occur; and toxicity may not be limited to stone formation in animal studies if melamine is present in high dosages or in combination with cyanuric acid.

DISCLOSURES

None.

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