

Background Paper on the Chemistry of Melamine Alone and in Combination with Related Compounds*

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Toxicological and Health Aspects of Melamine and Cyanuric Acid

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* The views presented in this article do not necessarily reflect those of the
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This chemistry background paper briefly summarizes key information on melamine related to past contamination incidents, uses, chemical structures and physicochemical properties (including the related triazines: cyanuric acid, ammeline and ammelide), manufacturing processes and product purity, complex formation and knowledge gaps.

1. GENERAL BACKGROUND ON CURRENT AND PAST MELAMINE CONTAMINATION INCIDENTS

Since September 2008, when the current melamine contamination incident became widely known, the World Health Organization (WHO) reported that there have been three infant deaths, and “more than 47,000 infants and young children in China were hospitalized for urinary problems, possible renal tube blockages and possible kidney stones related to the consumption of melamine contaminated infant formula and related dairy products” (WHO, 2008). China’s General Administration of Quality Supervision, Inspection and Quarantine conducted a national survey that revealed melamine contamination (from 0.1 to >2500 mg/kg) in powdered infant formulas produced by 22 Chinese companies (USFDA, 2008). Other countries have also reported detection of melamine in eggs (probably from contaminated feed) and milk-containing products manufactured in China, such as liquid milk, biscuits, candies, frozen yoghurt dessert and beverages. It appears that the contamination with melamine happened during milk production, where it had been intentionally added to raw milk at milk collection centres for at least 9 months (WHO, 2008).

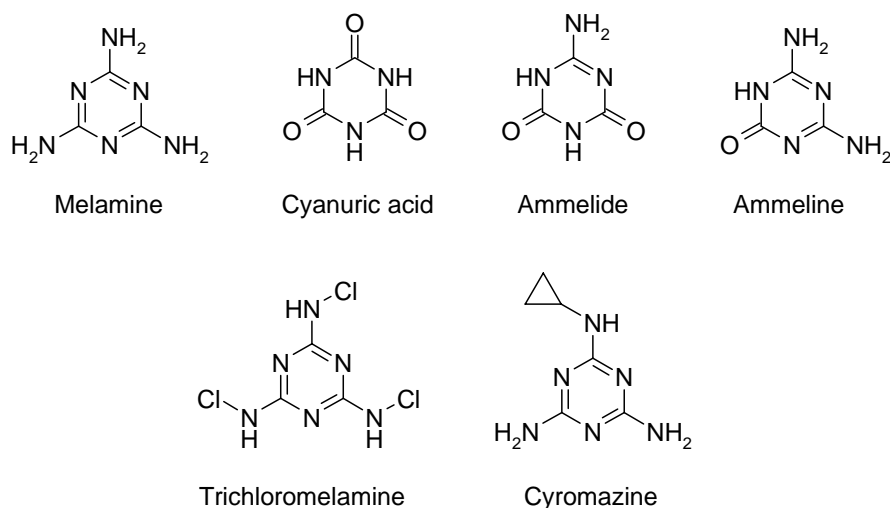
Several other melamine contamination incidents in pet food ingredients or products have been previously reported. Melamine has a high nitrogen content (66.6% by weight), which makes it attractive for economic adulteration as a fraudulent substitute for protein, especially when indirect protein assays based on total nitrogen are used. In 2007, melamine and related triazines were found at levels ranging from 0.8% to 26% in wheat gluten and rice protein extract imported from China that were used to manufacture pet food in the United States of America (USA) (Satzger, 2008). Pet food products contaminated by 0.001–0.7% melamine and related triazines (G. Diachenko, personal communication, 2008) led to the recall of 1154 pet food product types and are believed to have caused the deaths of more than 1000 dogs and cats due to kidney failure (Brown et al., 2007; Rovner, 2007; Rumbelha et al., 2007; Reimschuessel et al., 2008; Tolleson, 2008). Another incident of pet illnesses and acute renal failures occurred in several Asian countries during March 2004 due to pet food products manufactured at a single factory in Thailand (Brown et al., 2007).

Recent chemical and histological analyses of tissues from the 2004 and 2007 incidents by Brown et al. (2007) and by Thompson et al. (2008) have determined that the acute renal failures in animals from both pet food incidents were associated with the presence of melamine and cyanuric acid. In 1988, an Italian survey of fish meal products, which can be used in pet foods, produced from 1979 to 1987 reported melamine contamination ranging from 0.94% to 1.6% (Cattaneo & Ceriani, 1988). Stricter guidelines, increased surveillance, education and changed manufacturing procedures enabled a large decrease (from 72% to 5%) in the incidence of melamine-contaminated fish meal products in Italy (Cattaneo & Ceriani, 1988; Tolleson, 2008).

2. USES AND CHEMICAL AND PHYSICAL PROPERTIES OF MELAMINE AND RELATED COMPOUNDS

Melamine (Chemical Abstracts Service [CAS] No. 108-78-1) (see Figure 1 and Table 1) is a nitrogen-rich heterocyclic triazine used primarily in the synthesis of melamine-formaldehyde resins (MFR) for the manufacture of laminates, plastics, coatings, commercial filters, glues or adhesives, and moulding compounds (dishware and kitchenware) (Bizzari & Yokose, 2008). Melamine can also be used as a colorant and as a fertilizer; however, it is not approved for these uses in the USA. There are no approved uses for the direct addition of melamine to food in the USA. The only regulated use for melamine per se in the USA is as an adhesive under 21 Code of Federal Regulations (CFR) section 175.105. There are several regulations for MFR, including 21 CFR sections 175.300 (Resinous and polymeric coatings), 175.320 (Resinous and polymeric coatings for polyolefin films), 176.170 (Components of paper and paperboard in contact with aqueous and fatty foods), 176.180 (Components of paper and paperboard in contact with dry food), 177.1010 (Acrylic and modified acrylic plastics, semirigid and rigid), 177.1200 (Cellophane), 177.1460 (Melamine-formaldehyde resins in moulded articles), 177.1630 (Polyethylene phthalate polymers), 177.2260 (Filters, resin-bonded) and 177.2470 (Polyoxymethylene copolymer).

Figure 1. Structures of melamine and related triazine compounds



Trichloromelamine (CAS No. 7673-09-8) (see Figure 1), which decomposes to melamine, is regulated in the USA for use in sanitizing solutions used on food-processing equipment, utensils and other food contact articles, with the exception of milk containers or equipment (21 CFR section 178.1010). In addition, the United States Environmental Protection Agency (USEPA) allows for the use of trichloromelamine as a sanitizer and disinfectant on hard surfaces and as a component of a wash solution for fruit and vegetables.

Melamine is a metabolite of the pesticide **cyromazine** (CAS No. 66215-27-8) (see Figure 1). The USEPA (1999) indicated that only 10% of cyromazine is converted to melamine in vivo and has removed melamine from the tolerance limits as a residue of toxicological concern for cyromazine. The USEPA (1999) concluded that only residues of the parent compound, cyromazine, should have a listed tolerance level. However, it is known that cyromazine on the surface of fruits and vegetables is converted to melamine over time (Lim et al., 1990; USEPA, 1999).

Table 1. Physical and chemical properties of melamine and analogues

	Melamine	Cyanuric acid	Ammelide	Ammeline
Chemical formula	C ₃ H ₆ N ₆	C ₃ H ₃ N ₃ O ₃	C ₃ H ₄ N ₄ O ₂	C ₃ H ₅ N ₅ O
Molecular weight (g/mol)	126.12	129.07	128.09	127.10
% nitrogen (w/w)	66.6	32.6	43.7	55.1
Appearance	Fine white crystalline powder ^a	White crystalline solid ^b	White powder ^c	White powder ^c
Melting point (°C)	345–347 Decomposes ^{c-f}	360 ^{d,g}	Decomposes ^f	Decomposes ^f
Aqueous solubility (mg/l)	3240 ^e	2000 ^g	76.9 ^h	75 ⁱ
pK _a (dissociation constant)	5.35 (25 °C) ^d	4.74 (25 °C) ^d		9.65 (40 °C) ^c

^a Bizzari & Yokose (2008).

^b Budavari et al. (1996).

^c Bann & Miller (1958).

^d USFDA (2007).

^e ChemIDplus (2008a).

^f Lide (1991).

^g ChemIDplus (2008b).

^h ChemIDplus (2008c).

ⁱ ChemIDplus (2008d).

Cyanuric acid (CAS No. 108-80-5) (see Figure 1 and Table 1) is an oxytriazine melamine analogue that may be produced as a by-product in melamine synthesis. It is a USFDA-accepted component of feed-grade biuret, a ruminant feed additive, and is also found in swimming pool water as the dissociation product of dichloroisocyanurates used for water disinfection. When used for disinfection purposes in drinking-water, sodium dichloroisocyanurate is rapidly dechlorinated to cyanurate (Brady, Sancier & Sirine, 1963; Matte et al., 1990). Cyanuric acid derivatives are regulated in the USA as components of sanitizing solutions for use on food-processing equipment, utensils and other food contact articles (21 CFR section 178.1010).

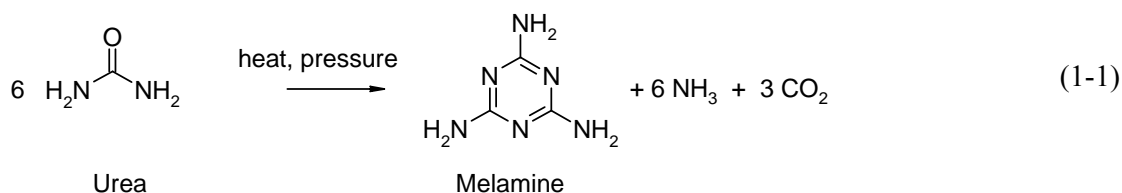
Ammelide (CAS No. 645-93-2) and **ammeline** (CAS No. 645-92-1) (see Figure 1 and Table 1) are, respectively, monoamino- and diaminoxytriazine analogues produced as by-products of melamine synthesis or by the microbial degradation of melamine (see section 2.3 below). Ammeline is used in lubricating greases (USFDA, 2007). No information on the uses of ammelide was available.

2.1 Manufacture

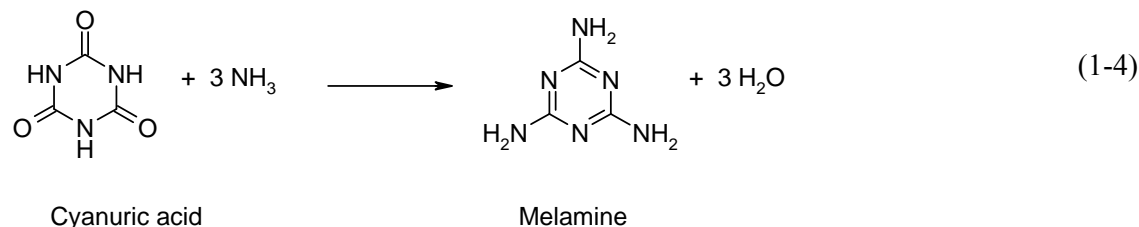
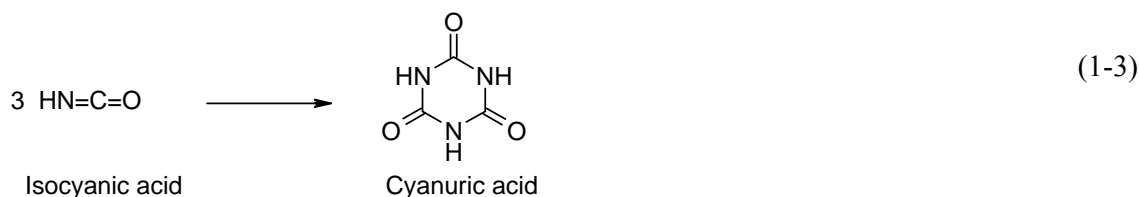
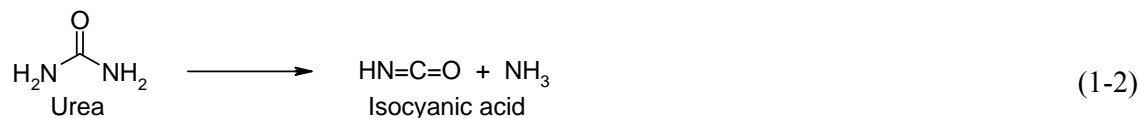
World production of melamine in 2007 was approximately 1.2 million tonnes, with the predominant producers being located in China and Western Europe (Bizzari & Yokose, 2008). Melamine can be produced from three different starting materials: urea, dicyandiamide or hydrogen cyanide. Commercially produced melamine is manufactured using urea as a starting material (Maxwell, 2007; Bizzari & Yokose, 2008). There are differences in the literature regarding the manufacture of melamine from dicyandiamide. Some sources indicate that commercial production of melamine from the thermal condensation of dicyandiamide ceased during the 1980s (Bizzari & Yokose, 2008). However, other sources indicate that this process is still used to manufacture melamine (Ono et al.,

1998). It does not appear that production of melamine from hydrogen cyanide is used commercially.

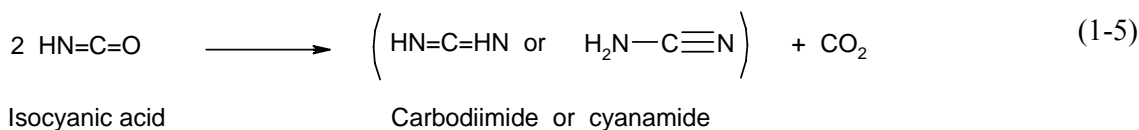
The net reaction for the production of melamine from urea is shown in Equation 1-1:

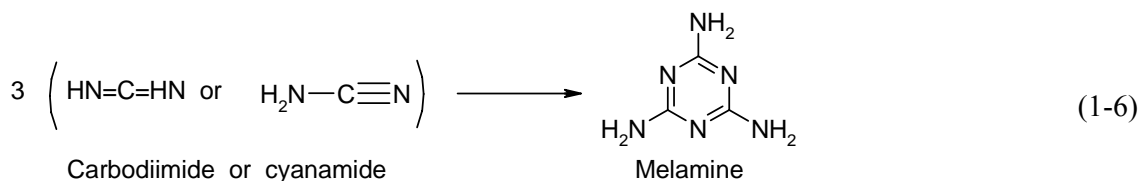


The reaction is typically carried out in one or more stages using either a high-pressure or a low-pressure process. The high-pressure process is performed in the liquid phase without a catalyst, at pressures of 90–150 bar and temperatures of 380–450 °C. In this process, urea is first converted to isocyanic acid (Equation 1-2), which then forms cyanuric acid (Equation 1-3). The cyanuric acid is then reacted with ammonia to form melamine (Equation 1-4).



The low-pressure process is carried out in the gas phase in the presence of a catalyst, such as modified aluminium oxide or aluminosilicate, at pressures of 1–10 bar and temperatures of 350–450 °C. As with the high-pressure process, urea is first converted to isocyanic acid (Equation 1-2). In the second stage of the reaction, the isocyanic acid is converted on the catalyst to either cyanamide or carbodiimide (Equation 1-5), which are then converted to melamine (Equation 1-6).



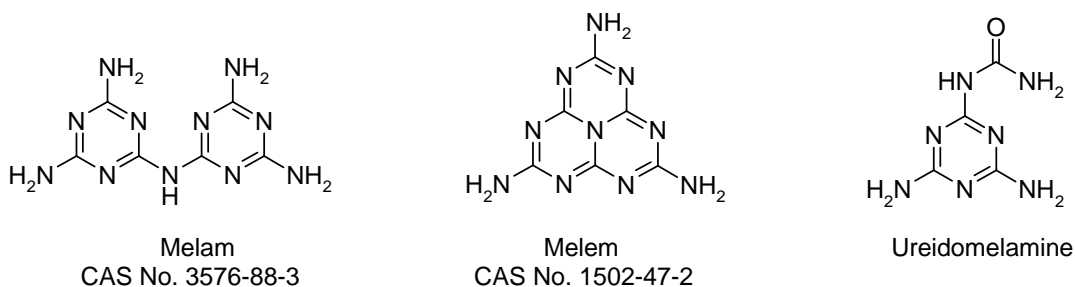


In general, the products from either the low- or high-pressure process are quenched with water or an aqueous mother liquor. With recycling of reaction by-products (ammonia and carbon dioxide), reaction yields can be as high as 95%. Purification can be accomplished by filtration, centrifugation or crystallization. There are also proprietary technologies based on the low-pressure method that yield product purities as high as 99% without further purification (Bizzari & Yokose, 2008).

2.2 Purity

The purity of melamine products is highly dependent upon the manufacturing process and the level of purification employed. It stands to reason that both high-purity and low-purity melamine are available. There is not a great deal of published information regarding the purity of melamine. One study (Ono et al., 1998) analysed four commercial melamine samples for the purpose of comparing high-performance cation-exchange chromatography with an unidentified analytical technique. The study found the following range of impurities in the four samples: melam¹ (not detected [nd]–0.2%), melem (nd–0.05%), ammeline (0.03–0.15%), ammelide (nd–0.05%) and ureidomelamine (nd–0.15%) (see Figure 2). It is unclear whether cyanuric acid was absent in these melamine samples or whether the techniques used were unable to detect it.

Figure 2. Additional melamine impurities: triazines and heptazines



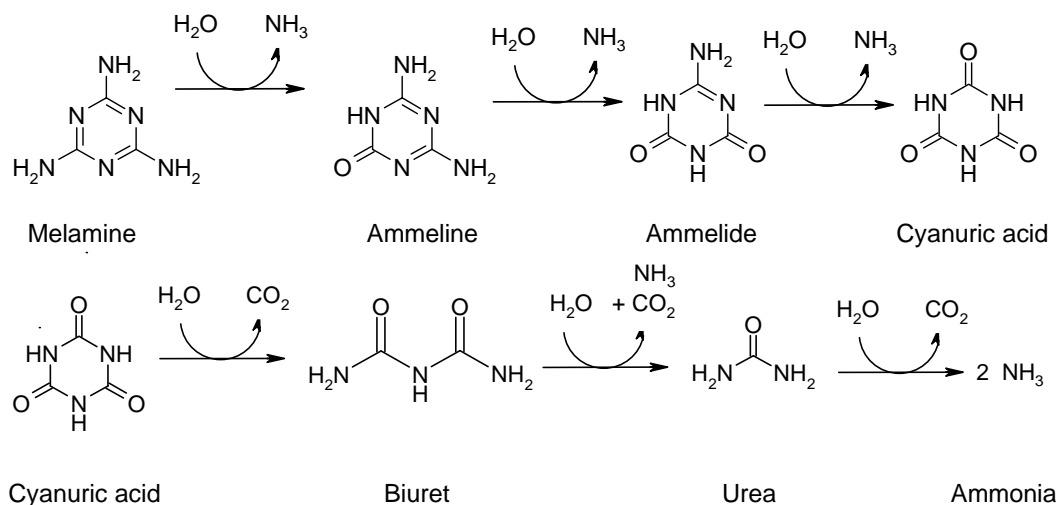
Melamine-containing solids can also be recovered from the mother liquor wastewater stream following the crystallization stage in the commercial production of melamine. The mother liquor wastewater stream can be concentrated to a solids content of 1.5–5% by weight (Lahalih & Absihalabi, 1989). Analysis of one such wastewater stream having a total solids percentage of 1.80% yielded melamine at 1.27% of the total solids, oxytriazines (including ammeline, ammelide and cyanuric acid) as 0.42% of the total solids and polycondensates, including melem, melam and melon ($\text{C}_6\text{H}_3\text{N}_9$)_x, as 0.012% of the total solids (Ono et al., 1998). It is possible that solids from the mother liquor wastewater stream are representative of the “melamine scrap” discussed in many news articles related to melamine adulteration.

¹ Different references use different spellings for “melam” and “melem”. In some instances, “melam” and “melem” are spelled “melame” and “meleme”, respectively. We believe these substances to be the same. Therefore, the names “melam” and “melem” are used throughout this document for consistency.

2.3 Degradation of melamine

It has been demonstrated that melamine can be metabolized by at least two strains of bacteria (*Pseudomonas* strain A and *Klebsiella terrigena*) into carbon dioxide and ammonia via the pathway shown in Figure 3 (Jutzi, Cook & Hutter, 1982; Shelton et al., 1997). As shown in the figure, melamine is metabolized through the successive deamination reactions to form ammeline, then ammelide, then cyanuric acid, with further breakdown to biuret, urea and, ultimately, ammonia and carbon dioxide.

Figure 3. Melamine metabolic breakdown pathway attributed to *Pseudomonas* strain A and *Klebsiella terrigena*



3. MELAMINE COMPLEXES: STRUCTURES AND PROPERTIES

Melamine and related triazine derivatives are able to form self-assembling, high molecular weight complexes via organized intramolecular networks of hydrogen bonds and π - π aromatic ring stacking (Seto & Whitesides, 1990; Whitesides, Mathias & Seto, 1991). Supramolecular triazine complexes are similar in several ways to the naturally occurring supramolecular complexes of the melanin pigments: similar dimensions; coplanarity of monomer units with π - π aromatic ring stacking; the availability of free amines, carbonyl groups and hydroxyls; and the ability to chelate transition metals.

The unique capabilities exhibited by melamine and related triazine derivatives provide useful molecular scaffolding components exploited by the field of supramolecular chemistry to produce a variety of sophisticated nano- or microscaled molecular complexes. Known triazine-based self-assembling supramolecular complexes include rosettes (Li, Chin & Whitesides, 1996; ten Cate et al., 2005), crinkled tapes (Bielejewska et al., 2001), nanoropes and nanoribbons (Yagai et al., 2007), propeller-like banana complexes (Barbera et al., 2006), molecular guest boxes (Kerckhoffs et al., 2005), channel structures (Thomas & Kulkarni, 2007), hydrogels (Saha, Manna & Nandi, 2007), supramolecular membranes (Kawasaki et al., 2001) and others (MacDonald & Whitesides, 1994). The hydrogen bond-forming properties of melamine, described below in further detail, allow it to interact with nucleobases, such as uracil (Thomas & Kulkarni, 2007), whereas those of cyanuric acid allow it to interact with purines, such as adenine (Gasparutto et al., 1999). The latter application involves its incorporation into oligonucleotides.

Although the chemical structures of melamine and related triazines are subject to tautomeric equilibrium (Hughes, 1941), spectroscopic studies (Klotz & Askounis, 1947; Ito, 1953) confirmed that the enamine form of melamine and the keto form of cyanuric acid are the resonance structures favoured in acidic to neutral solutions, consistent with the enthalpy difference required for hydrogen transfer from an amide to imide configuration ($\Delta H = 0$) compared with those for the more favourable enol to keto conversion ($\Delta H = -42$ kJ/mol).

Via the sp^2 hybridized nitrogen atoms of its triazine ring, each melamine monomer provides three unshared pairs of electrons available as hydrogen bond acceptors. In addition, unsubstituted melamine includes three exocyclic primary amines, each with the potential to provide a pair of hydrogen bond donors. A useful and simplified paradigm to appreciate the hydrogen-bonding pattern of melamine is that of an equilateral triangle, with each 4.8 Å side providing an offset triplet of hydrogen bond donor–acceptor–donor groups (D-A-D) available to form complementary intermolecular bonds with appropriately spaced functional group triplets comprising a hydrogen bond acceptor–donor–acceptor (A-D-A) motif (Arduini et al., 2003). Likewise, the hydrogen-bonding properties of cyanuric acid may also be appreciated in terms of another equilateral triangle, 4.6 Å per side, each composed of A-D-A hydrogen bond-forming groups that complement exactly those of melamine. In its keto configuration, cyanuric acid provides three N–H hydrogen bond donors via sp^3 hybridized nitrogen atoms of its triazine ring and six pairs of unshared electrons: two pairs each for the three carbonyl oxygen atoms. Each pair of unshared electrons is oriented within the plane of the triazine ring to act as hydrogen bond acceptors for properly oriented hydrogen bond donors. Typical enthalpies for intermolecular hydrogen bonds of the N–H \cdots N and N–H \cdots O types are -13 kJ/mol and -8.6 kJ/mol, respectively (Fessenden & Fessenden, 1979), potentially contributing -34 kJ/mol for each melamine–cyanuric acid interface. Triazine aromatic π - π ring stacking (3.6 Å nominal spacing) is estimated to contribute -13 kJ/mol to the binding interaction (Bates et al., 2008).

As described above, the D-A-D hydrogen bond-forming interface of melamine complements ideally the imide-type A-D-A hydrogen bond-forming interface of cyanuric acid (Figure 4). Biomolecules possessing similar cyclic imide A-D-A hydrogen-bonding domains are common. Uracil, riboflavin, barbituric acid and uric acid each possess imide groups known to interact with melamine in self-associating complexes (see Figure 5 and references in the second paragraph of this section). Although unconfirmed experimentally, other naturally occurring biomolecules, such as thymine, xanthine, the allopurinol metabolite oxypurinol and the uric acid metabolite allantoin, each include cyclic imide moieties with the potential to form multiple hydrogen bonds with melamine.

Figure 4. Melamine (D-A-D) and cyanuric acid (A-D-A) units form hydrogen-bonded networks that self-assemble into supramolecular complexes

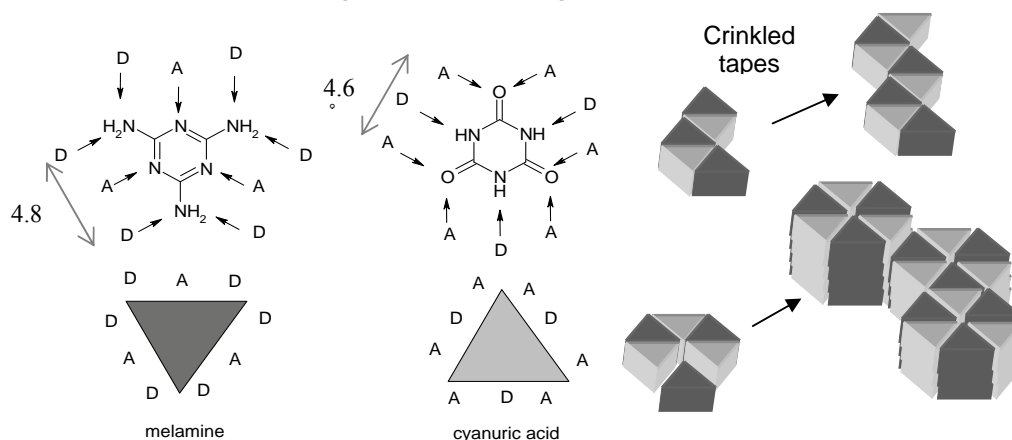
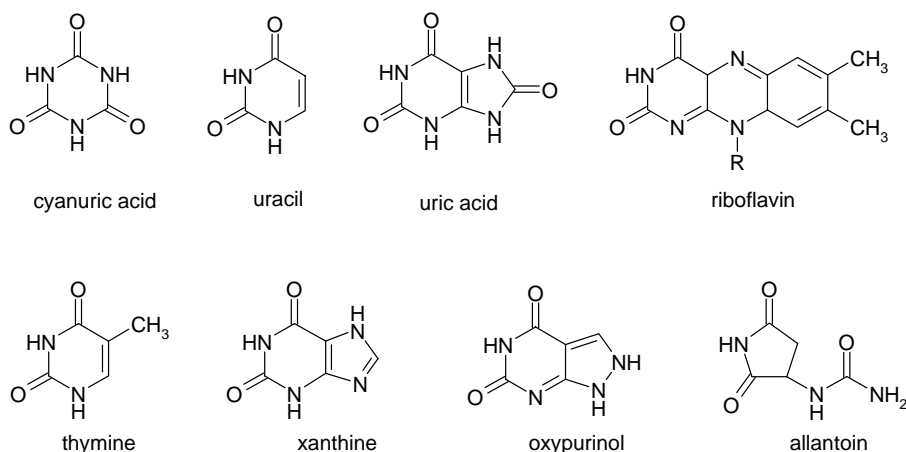


Figure 5. Putative or known ligands for melamine and cyanuric acid found in urine



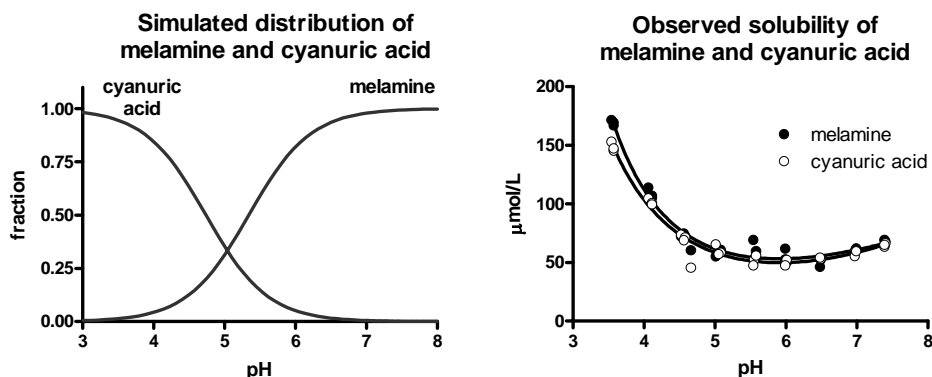
Unlike the intermolecular complexes involving melamine and cyanuric acid, intermolecular complexes with ammeline and ammelide have not been studied in similar detail, nor have thorough spectroscopic studies evaluated their resonance structures. By analogy to the favoured resonance structure for melamine, it can be hypothesized that ammeline, with two exocyclic amines and one exocyclic oxygen atom, possesses one melamine-like D-A-D hydrogen-bonding interface and two hybrid D-A-A/D interfaces composed of an exocyclic amine (D), an unshared pair of electrons on an sp^2 hybridized ring nitrogen (A) and either an unshared pair of electrons on the exocyclic oxygen (A) or the bound hydrogen (D) of its hydroxyl group. Similarly to the keto form of cyanuric acid, ammelide should exhibit one cyanuric acid-like A-D-A hydrogen-bonding imide-type interface and a pair of hybrid A/D-D-A interfaces. Although ammeline and ammelide might be expected to exhibit greater promiscuity in the formation of hydrogen bonds with other triazines, this characteristic may come at the cost of weaker intermolecular bonds, as shared electrons associated with sp^2 hybridized exocyclic oxygen atoms are further delocalized through resonance.

Triazine hydrogen bond networks may be subject to disruption by acid–base equilibria or high temperature. Low molecular weight chemical species with high water solubility and the ability to form competitive hydrogen bonds, such as urea, guanidine, amines, alcohols and dimethyl sulfoxide, are generally considered inadequate to overcome the sum of intermolecular forces binding complexes of melamine and cyanuric acid. Alternatively, triazine hydrogen bond networks can be blocked by reagents that adduct hydrogen bond donors or acceptors covalently. For example, the primary amines of melamine are susceptible to form Schiff's base adducts with formaldehyde, simultaneously eliminating hydrogen bond donor groups and disrupting the critical intermolecular spacing required for the proper alignment of the remaining hydrogen bonds.

The effect of pH on the solubility of the melamine–cyanurate hydrogen-bonded complex was recently determined (Figure 6) (Tolleson, 2008). As predicted from the acid dissociation constants for the melaminium cation ($C_3H_7N_6^+$) and cyanuric acid, minimum solubility for the complex was observed under conditions favouring the co-existence of melamine free base and non-ionized cyanuric acid (pH 5.0). The solubility of the complex increased markedly as the pH decreased to pH 3.5 and the concentration of melaminium cation increased. By contrast, the solubility of melamine–cyanurate increased marginally as the pH increased to pH 7.5. Interestingly, spherulite crystals of melamine–cyanurate formed

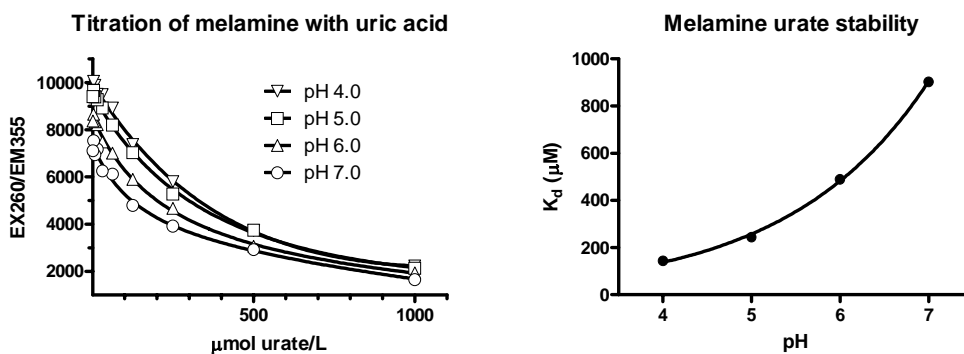
within the urinary tracts of fish and pigs have been shown to dissolve in formalin over a period of hours but in acid in a period of minutes (Reimschuessel et al., 2008).

Figure 6. Influence of pH on solubility of melamine–cyanurate



Because melamine exhibits intrinsic fluorescent properties (excitation maximum 255 nm, emission maximum 390 nm), melamine titrations can be monitored fluorimetrically. Using this approach, the melamine–cyanurate complex exhibited a K_d of $31 \pm 7 \mu\text{mol/l}$ in water (W. Tolleson, personal communication, 2008). For comparison, the transition metal ions Cu(II), Fe(II) and Zn(II) exhibited much weaker binding to melamine (K_d 1.8, 1.0 and 30 mmol/l, respectively). Fluorescence titrations were also used to evaluate the effect of pH on the stability of melamine–uric acid complexes (Figure 7). At neutral pH, the affinity of melamine for uric acid was found to be 29-fold weaker than that for cyanuric acid. Melamine–urate exhibited up to 6.3-fold tighter binding under acidic conditions in comparison with its affinity at pH 7.0 (K_d 140, 240, 490 and 900 $\mu\text{mol/l}$ at pH 4.0, 5.0, 6.0 and 7.0, respectively). Chickens, which normally excrete larger amounts of uric acid than mammals, were exposed to melamine in preliminary USFDA studies. These studies showed that spherulites, presumably composed of melamine–urate crystals, dissolved rapidly in formalin fixative (R. Reimschuessel, personal communication, 2008).

Figure 7. Influence of pH on melamine–urate dissociation



4. KNOWLEDGE GAPS AND RECOMMENDED STUDIES

It has been established that oral exposure of diverse animal species (cats, dogs, rats, pigs and fish) to a combination of melamine and cyanuric acid in feed (2.5–400 mg/kg in various studies) produces calculi that obstruct renal tubules, leading to acute renal failure

(Puschner et al., 2007; Dobson et al., 2008). However, incomplete knowledge is available regarding the threshold dose (no-observed-adverse-effect level [NOAEL]) for triazine-induced renal failure. To address this important knowledge gap, the United States National Toxicology Program and the USFDA recently approved laboratory studies in rats, miniature pigs and swine to determine the NOAEL for melamine and cyanuric acid (G. Gamboa da Costa and R. Reimschuessel, personal communications, 2008).

The variable or unknown composition of triazine-contaminated food products complicates both risk assessment models and the design of potential treatment options. The nature of the chemical co-contaminants present with melamine in foods is perhaps the most significant unsettled issue relevant to the formation of insoluble deposits within the urinary tract that result in renal impairment. A thorough inventory is needed of likely precipitate-forming co-contaminants with melamine present in adulterated food items, along with laboratory studies to determine the threshold concentrations required for formation of such precipitates under conditions found in the urinary tract.

Analytical surveys performed during the 2007 pet food and 2008 milk product contamination episodes implicate the typical by-products of melamine synthesis and degradation (e.g. various oxytriazine and heptazine species) as the agents most consistently detected with melamine that are also present at comparable levels (see section 2.1). Notably, the composition of “melamine scrap” probably represents a wide range of possible mixtures, with the presence of cyanuric acid highly likely, as a result of either incomplete reactions during production or a concentration effect concurrent with removal of melamine during purification. If industrial-grade melamine has been stored outdoors, as suggested in a few cases, it would be subject to an uncertain sequence of biological degradation, potentially yielding oxytriazines able to form stable melamine complexes (see section 2.3). Although lacking scientific verification, recent news reports have further alleged that additional, non-triazine contaminants were sometimes introduced into milk products along with melamine. It has been reported that melamine-based “protein powders” were formulated with other chemicals, particularly maltodextrin, to increase solubility and palatability. It has also been suggested that antibiotics, preservatives (e.g. potassium nitrate or sodium nitrite), formaldehyde, hydrogen peroxide, soya powder, whey or vegetable oil may also have been added. If melamine was added to increase the apparent nitrogen content of food ingredients to enhance their market value, then it is conceivable that similar rapacious motives could encourage the use of alternative nitrogen-rich compounds, such as urea, ammonium salts or guanidium salts.

In addition to complexes with exogenous contaminants, melamine and other triazines possess the potential to form hydrogen-bonded complexes with endogenous biochemicals (see section 3). Cyclic imide biochemicals (e.g. uric acid, xanthine, uracil, thymine, riboflavin, oxypurinol and allantoin) are transported through the circulation, are present in the initial glomerular filtrate and are presumed capable of forming hydrogen-bonded networks with melamine.

The potential for insoluble melamine precipitates to form under conditions found in the urinary tract from those endogenous or exogenous chemical species available at adequate levels should be evaluated; the solubilities of these complexes should be measured under those conditions. The most urgent emphasis should be placed on characterizing the properties of complexes formed between melamine and the oxytriazine species detected in contaminated foods (cyanuric acid, ammelide and ammeline). Some anecdotal descriptions of “sludge-like” or “sand-like” urinary precipitates observed in infants exposed to melamine-contaminated milk products are considered reminiscent of certain uric acid-containing uroliths that could possibly contain melamine. Both low pH and ammonium ions are known to reduce the solubility of urate in the urine, with unknown effects on the solubility of melamine. The

solubility and binding properties of melamine–urate complexes represent another important research priority.

The chemical composition of renal calculi found in victims of melamine intoxication is an issue of critical importance. Rational medical intervention to resolve renal obstruction in a patient requires knowledge of the chemical identity and properties of the insoluble matter that may be unique to the urinary tract of that individual. Quantitative analyses of the soluble components of urine may also provide important diagnostic information required for effective patient care.

The properties of laboratory crystals formed from melamine and cyanuric acid in the presence of serum or urine appear similar to those of renal calculi observed in pets or other animal species exposed to triazine-contaminated feed. The artificial renal calculi formed in the presence of biofluids are distinct from white needle-like crystalline precipitates of melamine and cyanuric acid produced in water or aqueous buffer solutions. The implication is that biomolecules, such as proteins or protein fragments present in urine, are involved in the organization of melamine–cyanurate to form the characteristic 10–100 μm yellow to brown coloured, birefringent, fluorescent spherulite calculi associated with the melamine-induced renal failure syndrome. Protein analysis should be performed on melamine-containing uroliths to elucidate whether urinary proteins play a role in crystal formation, growth, adhesion to renal tubule epithelial cells or solubility. Serum proteins identified consistently in melamine–cyanurate crystals formed in vitro include serum albumin, haemoglobin A chain fragments and apolipoproteins (Tolleson, 2008).

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