DOI: 10.5281/zenodo.33513

Food Packaging Forum

Dossier - Melamine

March 2013 **Birgit Geueke**

1 Physical and chemical properties

Melamine (CAS 108-78-1; IUPAC name: 1,3,5-triazine-2,4,6-triamine) is a heterocyclic aromatic compound with the chemical formula $C_3H_6N_6$ (**1**, Figure 1). It is sold as white, powdered crystal and its solubility is 5, 0.06, 0.03, and 0.01 g/L in water, ethanol, acetone, and dimethylformamide, respectively, at 30°C [1]. Melamine has an octanol/water partition coefficient (log K_{OW}) of -1.37 [2].

2 Production and use

First industrial synthesis routes for melamine used dicyandiamide as starting material, but by the end of the 1960s this process was replaced by an alternative using urea. Six molecules of urea form one equivalent of melamine under the release of three carbon dioxide and six ammonia molecules. The process runs at 400°C either under low pressure using aluminum catalysts or at high pressure without catalysts. Depending on the pressure, melamine is produced as gas or liquid. Byproducts that are formed during production include melam, melem, and melone, as well as oxotriazines such as ammeline (**2**), ammelide (**3**), and cyanuric acid (**4**), but the commercially available melamine generally has purities of >99.9%.

Melamine is used as monomer in the production of melamine resin (or melamine formaldehyde; CAS 9003-08-1) (**5**, Figure 1). One molecule melamine generally reacts with three formaldehyde molecules at basic pH values under the formation of hydroxymethylmelamines. These molecules are cross-linked at high temperatures and react with polyester, acrylics and epoxides. A look into the catalogue of Cytec, a chemical company specialized in coatings, helps to get a first overview on the diversity of different melamine-containing coatings [3].

In general, melamine resin is used to produce two different types of food contact articles:

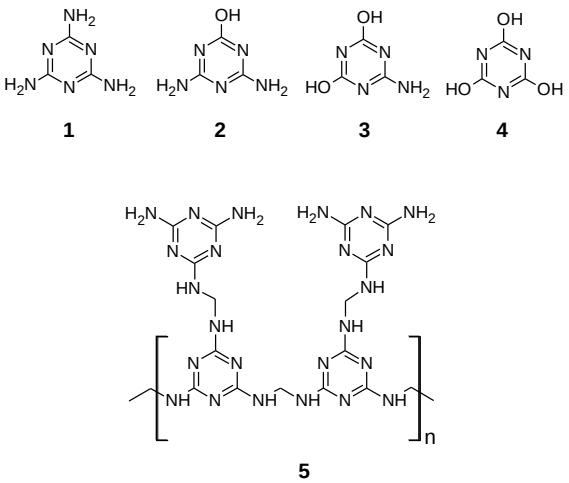

Figure 1. Chemical structures of melamine **1**, ammeline **2**, ammelide **3**, cyanuric acid **4,** and melamine resin **5**.

- Modified and cross-linked melamine resins are used as surface coatings for paper, board, beverage cans and jar lids [4, 5]. Additionally, these materials are applied in the production of automotive body panels and household appliances (Figure 2, yellow).
- Melamine resins are also blended with cellulose fillers, pigments and other additives to form molding compounds for the productions of dinnerware, chopsticks and electrical equipment (Figure 2, orange). The unbreakable kitchenware and plates find special applications in nurseries and camping.

Furthermore, melamine adhesives are applied in the furniture and construction industries to form boards, beams and wood-based panels. Different melamine preparations are used in the production of laminate floorings. Further applications include banknotes, printed textiles, concrete additives and flame retardants.

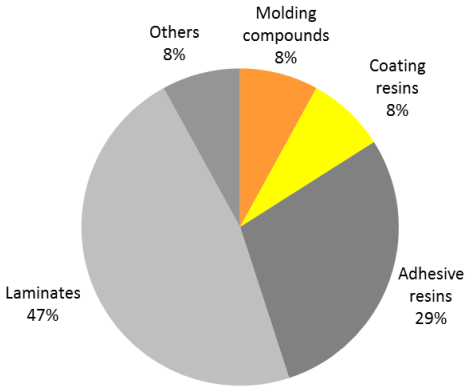

Figure 2. Applications of melamine (Data were derived from [6]). Melamine-containing FCMs either belong to the molding compounds (orange) or coating resins (yellow).

3 Market data

Melamine belongs to the high-production volume chemicals. In 2004, the global production capacity was about 1 million tons per year [6, 7] (Table 1). Estimates from previous decades showed lower global production capacities of 200,000 and 610,000 tons per year in 1970 and 1994, respectively. These data were derived from the peer-reviewed Hazardous Substances Databank of the National Library of Medicine [7]. The production capacity in the US was around 100,000 tons per year in the nineties and decreased to 80,000 tons per year in this century (Table 2). From the data presented in Figure 2, we assume that less than 10% of the total melamine production volume is used as food contact materials.

Table 1. Global production capacity of melamine.

Year	Global capacity (1000 tons/year)	Reference
1994	520 / 610	[6] / [7]
1998	660	[6]
2003	900	[6]
2004	990 / ≈1,000	[6] / [7]

Table 2. US production capacity of melamine.

Year	US capacity (1000 tons/year)	Reference
1991	97	[7]
1997	102	[8]
2006	80	[7]
2009	80	[9]
2012	80	[10]

4 Historical dates

- In 1834, melamine was prepared by Justus Liebig. 51 years later its chemical structure was elucidated by A.W. von Hoffmann [1].
- In the late 1930s, the two companies Henkel & Co. and American Cyanamid developed industrial processes for the production of melamine resins. In the 1950s and 60s, melamine tableware was widely used in households and also restaurants, but it became less fashionable in the following decades. Nevertheless, melamine resin is still frequently used in tableware for specific purposes such as camping and nursery.
- In 2004, at least 6 000 cats and dogs died of renal failure in Southeast Asian countries. Four years later, this epidemic was related to melamine-contaminated pet food produced at a Thai factory.
- In 2007, pet food that was adulterated with melamine and cyanuric acid, and possibly ammeline and ammelide, was recalled from the US market after serious illness and deaths of thousands of dogs and cats [11].
- In 2008, melamine was measured in milk and infant formula in China. In particular, milk powders of the brand *Sanlu* contained high levels of melamine (150-4700 mg/kg) [12]. Melamine that is composed of 67% nitrogen by molecular weight was added to imitate higher protein concentrations in the adulterated milk. As a result, nearly 300 000 people became ill, 50 000 infants were hospitalized and six of them died [13].

5 Current regulations and risk assessment

The 2008 milk scandal in China showed the possible large health impacts of melamine. As a consequence, the WHO convened an expert meeting and published a report on the toxicological and health aspects of melamine and cyanuric acid in 2009 [12]. On the basis of two 13-week studies in rats [14], which were administered melamine in the diet, the lower limit on the benchmark dose for a 10% response rate ($BMDL_{10}$) was calculated to be 35 mg/kg body weight/day [12]. The toxicological endpoint selected in this study was the development of urinary bladder stones. The Tolerable Daily Intake (TDI) of 0.2 mg/kg body weight/day was calculated by dividing the $BMDL_{10}$ by a safety factor of 200. The safety factor is derived from the intra- and interspecific variability (factor 100) combined with a further uncertainty factor of 2 that accounts for the potential increased sensitivity of infants and data uncertainties.

In the European Union, melamine is authorized for use as monomer and additive in food contact plastics according to regulation EU 10/2011, but its derivatives cyanuric acid, ammelide and ammeline are not on the EU positive list. Its widespread use as monomer is described in the section “Production and use”, whereas only one possible application as additive is known [15]. In 2010, EFSA reduced the Tolerable Daily Intake (TDI) from 0.5 to 0.2 mg/kg body weight/day following the WHO recommendations [15]. As a consequence, the Specific Migration Limit (SML) was also reduced from 30 to 2.5 mg/kg food in 2011 (Commission Regulation 1282/2011). The TDI of 0.2 mg/kg is not applicable if there is significant concomitant exposure to cyanuric acid, ammelide or ammeline, because the risk of urinary crystal formation is highly increased [15]. Cyanuric acid is on the on the Swiss inventory list of substances for printing inks [16].

In the US, melamine and melamine formaldehyde resins are authorized as adhesives and components of coatings (21CFR, §175). Latter is additionally approved as paper and paperboard component (21CFR, §176), and as polymer and copolymer (21CFR, §177). In 2007, the FDA set a TDI of 0.63 mg/kg body weight/day on the basis of a 13-week rat study. The observed NOAEL of 63 mg/kg body weight/day was divided by a safety factor of 100. In 2008, the TDI was decreased further from 0.63 to 0.063 mg/kg body weight/day, because a 2007 study indicated increased toxicity from combined exposure to melamine and cyanuric acid [17]. This finding has also been supported by more recent studies [18, 19].

6 Toxicity of melamine

6.1 General toxicity

Melamine has a relatively low general toxicity with an LD_{50} of 3.2 g/kg in rats [16]. Only low cytotoxicity of melamine was observed *in vitro* when it was tested in kidney epithelial cell lines of cats and dogs [20]. In contrast, more recent *in vitro* assays using human kidney cell lines showed a variety of molecular and cellular effects in the presence of melamine. These effects could all lead to renal tubular cell injury via apoptosis, inflammation, and fibrosis at concentrations up to 1000 µg/mL [21]. No genotoxicity was shown for melamine in *in vitro* and *in vivo* tests [5]. The NOAELs for fetal and maternal toxicity in rats are about 400 and 1060 mg/kg body weight/day, respectively [12][1]. The International Agency for Research on Cancer (IARC) evaluated the carcinogenicity of melamine in 1999 [22]: Melamine produced urinary bladder tumors in male rats by a non-DNA-reactive mechanism and only under conditions in which calculi were produced. It was not classifiable as to its carcinogenicity to humans (Group 3).

6.2 Nephrotoxicity

Dalal and Goldfarb concluded in 2011 that “melamine was an unknown substance to nephrologists until very recently” [23], but the pet food and milk scandals initiated detailed investigations on the effects of melamine-contaminated food onto animal and human health with a strong focus on the renal system. The adulterated pet food not only contained melamine, but also cyanuric acid and possibly ammeline and ammelide, whereas the infant milk was solely contaminated with melamine. Nevertheless, the deaths and illnesses of infants and animals were all due to renal failure that was caused by crystal formation in the urinary system with some associated evidence for kidney toxicity. The calculi that were isolated from the urine of Chinese infants who drank melamine-contaminated milk were composed of melamine and uric acid with varying molar ratios (from 1:2 to 2.1:1) [24]. Ultrasonographic imaging studies showed that the melamine-related stones were found in the renal calyces, pelvis, the ureters, the bladder and the urethra [25, 26]. The presence of the calculi led to obstructive renal failure in some of these infants [24].

After the pet food scandals, experimental studies clearly showed that melamine has increased toxicity when it is fed in combination with cyanuric acid [17-19]. Animals fed with a combination of both chemicals developed severe renal dysfunction due to the formation of stones that consisted of melamine-cyanuric acid complexes. This

[1] The WHO report cites these numbers. We do not have access to the original file: Helwig J, Gembrandt C, Hildebrandt B (1996). Melamine—Prenatal toxicity in Wistar rats after oral administration (diet). Ludwigshafen, BASF AG, Department of Toxicology (Project No. 32R0242/94007).

observation is in accordance with the renal histopathological changes observed in dogs, cats and pigs that were accidentally fed with the contaminated food in 2004 and 2007 [23].
A very recent study reports that melamine is converted to cyanuric acid by the bacterial strain *Klebsiella terrigena* that can be present in the gut of mammals [27]. It demonstrates that microbial activities can affect and even increase the toxicity of food contaminants.
Wu et al. found out that individuals with stones in the renal system (both uric acid and calcium urolithiasis) had significantly higher urinary melamine concentrations than controls [28]. It has been hypothesized that chronic exposure to low levels of melamine may lead to the formation of kidney stones in adults, but further research is needed [29].

6.3 Further effects

Very recent reports include further toxicological effects of melamine. An *in vivo* study describes adverse effects on the male reproductive system of mice at dosages between 2 and 50 mg/kg body weight/day [30]. These effects include histopathological changes in the testes, abnormal sperm morphology and an increase in the apoptotic index of spermatogenic cells when the mice were treated with 50 mg/kg body weight/day. Yang et al. showed that the behavior of 2-3 month-old rats was slightly influenced by single melamine doses of 5 and 25 mg/kg body weight [31]. Additionally, the synaptic plasticity of hippocampal slices was clearly affected by melamine at bath concentrations of 50-500 μg/mL. In 2010, it was reported that melamine affects the potassium currents in rat hippocampal neurons *in vitro* at concentrations that were below the safe blood concentration of 0.5 μg/mL as proposed by the US Food Safety and Inspection Service [32, 33]. The authors suggest that this effect could explain melamine-induced neurotoxicity. An et al. hypothesized that melamine induces oxidative damage in the hippocampus of rats and that it damages the cholinergic system [34, 35]. Melamine was fed at a dose of 300 mg/kg per day for four weeks and, subsequently, behavioral, histological and biochemical tests were performed. The authors add oxidative damage as one further possible reason for melamine`s neurotoxicity.
Biochemical investigations report inhibition of heme peroxidases by melamine [36]. Analyses of the urinary metabolome of rats after administration of melamine (600 mg/kg body weight/day) and melamine-cyanuric acid mixtures (50 mg melamine and 50 mg cyanuric acid/kg body weight/day) resulted in disrupted amino acid metabolism including tryptophan, polyamine, and tyrosine metabolism, and altered TCA cyle and gut microflora structure [37].

7 Migration, exposure and biomonitoring

The WHO report distinguishes two exposure scenarios: (1) “Baseline” levels of melamine in food that result from migration and/or the food from accepted uses and (2) “adulteration” levels, which refer to the intentional addition of melamine to food to pretend higher protein concentrations [12]. Further dietary sources of melamine are degradation products of the pesticide cyromazine and disinfectants such as trichloromelamine. Although the toxicological effects of high doses of melamine were considered in the previous paragraphs, in this section we will only focus on the baseline levels of melamine that are a result of the consumption of non-adulterated food.
Melamine migrates from can coatings into food, probably due to decomposition of the coating [4]. The migration is strongly influenced by the temperature of the heat treatment and less influenced by the time of heating and the acidity of the food. Bradley et al. observed migration levels up to 332 μg/kg food [4]. These results are in agreement with a previous study in which the migration of melamine from melamine tableware into food and food simulants was tested: high temperature and also long heating times promote the migration of melamine, but the type of food does not have such a strong influence [38]. Chien et al. confirmed the temperature dependency of migration and they even observed melamine migration at 30 and 40°C [39]. In general, it is likely that the concentration of melamine in food caused by migration is less than 1 mg/kg food [12]. A small biomonitoring study with twelve participants showed that urinary melamine levels were statistically significantly increased in participants eating from melamine plastic table ware in comparison to ceramic bowls. Study participants excreted more than 8 μg of melamine (mean values) after consumption of 0.5 L hot noodle soup from melamine bowls within the next 12 hours [40]. The findings strongly suggest that melamine migrated from the tableware into the hot food even after short contact times.

8 Metabolism and biodegradation

Melamine undergoes renal clearance, is excreted in the urine of mammals, and does not accumulate. It has a relatively low half-life of 4.9 hours in rats [41] and 4 hours in pigs [42]. In humans, the estimated half-life of urinary melamine elimination was approximately 6 hours [40]. These two factors, the renal clearance and the low half-life, might cause the organ specific toxicity of melamine that is related to the excretory route. A study by Zheng et al. demands caution, because bacterial strains in the gut can convert melamine into cyanuric acid, thus increasing the potential of stone formation [27]. A rat study showed that melamine passes the placenta and is transferred to breast milk [43]. Melamine is not easily degradable in activated sludge and it might even inhibit bacterial growth resulting in decreased effluent water quality [44].

Abbreviations

FCM	Food Contact Material
FDA	Food and Drug Administration
LD_{50}	Lethal Dose, 50%
NOAEL	No Observed Adverse Effect Level
TCA	Tricarboxylic Acid Cycle
TDI	Tolerable Daily Intake
SML	Specific Migration Limit

References

1. Crews GM, Ripperger W, Kersebohm DB, et al. 2006. Melamine and guanamine. In: Ullman's Encyclopedia of Industrial Chemicals. K. Othmer, ed. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. pp 377-92.
2. Hansch C, Leo A, and Hoekman D. 1995. Exploring QSAR: Hydrophobic, electronic, and steric constants. ACS, Washington D.C.
3. Cytec. 2013. Can coating. [https://www.cytec.com/liquids/Downloads/Can%20Coatings_EMEA_En.pdf]
4. Bradley EL, Castle L, Day JS, et al. 2011. Migration of melamine from can coatings cross-linked with melamine-based resins into food simulants and foods. Food Addit Contam A. 28:243-50.
5. EFSA, CONTAM and CEF. 2010. Scientific opinion on melamine in food and feed. [http://www.efsa.europa.eu/de/efsajournal/doc/1573.pdf]
6. DSM Investor Relations. 2004. DSM Melamine - Growing a profitable business. [http://www.dsm.com/content/dam/dsm/cworld/en_US/documents/dijkman-24-sept.pdf]
7. US NLM. 2013. Hazardous substances databank (HSDB). [http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB]
8. Westervelt R. 1997. Melamine chemical cuts production. Chemical Week. 159:19.
9. Anonymous. 2009. Melamine. ICIS Chemical Business. 276.
10. Zaworski F, and Mirasol F. 2012. Melamine. ICIS Chemical Business. 281:42.
11. US FDA. 2007. Melamine pet food recall of 2007. [http://www.fda.gov/AnimalVeterinary/SafetyHealth/RecallsWithdrawals/ucm129575.htm]
12. WHO. 2009. Toxicological and health aspects of melamine and cyanuric acid. [http://www.who.int/foodsafety/publications/chem/Melamine_report09.pdf]
13. Chen JS. 2009. A worldwide food safety concern in 2008 - melamine-contaminated infant formula in China caused urinary tract stone in 290,000 children in China. Chin Med J (Engl). 122:243-4.
14. NTP. 1983. Carcinogenesis bioassay of melamine (CAS No. 108-78-1) in F344/N rats and B6C3F1 mice (feed study). [http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr245.pdf]
15. EFSA, CEF. 2006. Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food on a request from the Commission related to 2,2-bis(4-hydroxyphenyl)propane (Bisphenol A). [http://www.efsa.europa.eu/en/efsajournal/doc/428.pdf]
16. BAG. 2011. Packaging inks. [http://www.bag.admin.ch/themen/lebensmittel/04867/10015/index.html?lang=en/]
17. Puschner B, Poppenga RH, Lowenstine LJ, et al. 2007. Assessment of melamine and cyanuric acid toxicity in cats. J Vet Diagn Invest. 19:616-24.
18. Kim TH, Ahn MY, Lim HJ, et al. 2012. Evaluation of metabolomic profiling against renal toxicity in Sprague-Dawley rats treated with melamine and cyanuric acid. Arch Toxicol. 86:1885-97.
19. Reimschuessel R, Evans ER, Stine CB, et al. 2010. Renal crystal formation after combined or sequential oral administration of melamine and cyanuric acid. Food Chem Toxicol. 48:2898-906.
20. Dobson RL, Motlagh S, Quijano M, et al. 2008. Identification and characterization of toxicity of contaminants in pet food leading to an outbreak of renal toxicity in cats and dogs. Toxicol Sci. 106:251-62.
21. Hsieh TJ, Hsieh PC, Tsai YH, et al. 2012. Melamine induces human renal proximal tubular cell injury via transforming growth factor-β and oxidative stress. Toxicol Sci. 130:17-32.
22. IARC. 1999. Melamine - Summaries & evaluations. [http://www.inchem.org/documents/iarc/vol73/73-12.html]
23. Dalal RP, and Goldfarb DS. 2011. Melamine-related kidney stones and renal toxicity. Nat Rev Nephrol. 7:267-74.
24. Sun Q, Shen Y, Sun N, et al. 2010. Diagnosis, treatment and follow-up of 25 patients with melamine-induced kidney stones complicated by acute obstructive renal failure in Beijing Children's Hospital. Eur J Pediatr. 169:483-9.
25. Lam CW, Lan L, Che X, et al. 2009. Diagnosis and spectrum of melamine-related renal disease: plausible mechanism of stone formation in humans. Clin Chim Acta. 402:150-5.
26. He Y, Jiang GP, Zhao L, et al. 2009. Ultrasonographic characteristics of urolithiasis in children exposed to melamine-tainted powdered formula. World J Pediatr. 5:118-21.
27. Zheng X, Zhao A, Xie G, et al. 2013. Melamine-induced renal toxicity is mediated by the gut microbiota. Sci Transl Med. 5:172ra22.
28. Wu CF, Liu CC, Chen BH, et al. 2010. Urinary melamine and adult urolithiasis in Taiwan. Clin Chim Acta. 411:184-9.
29. Liu CC, Wu CF, Chen BH, et al. 2011. Low exposure to melamine increases the risk of urolithiasis in adults. Kidney Int. 80:746-52.
30. Yin RH, Wang XZ, Bai WL, et al. 2012. The reproductive toxicity of melamine in the absence and presence of cyanuric acid in male mice. Res Vet Sci. online.
31. Yang Y, Xiong GJ, Yu DF, et al. 2012. Acute low-dose melamine affects hippocampal synaptic plasticity and behavior in rats. Toxicol Lett. 214:63-8.
32. Yang JJ, Tian YT, Yang Z, et al. 2010. Effect of melamine on potassium currents in rat hippocampal CA1 neurons. Toxicol In Vitro. 24:397-403.
33. USDA. 2007. Disposition of hogs and chickens from farms identified as having received petfood scraps contaminated with melamine and melamine-related compounds and offered for slaughter. [http://docs.regulations.justia.com/entries/2007-05-30/07-2649.pdf]
34. An L, Li Z, Yang Z, et al. 2012. Melamine induced cognitive impairment associated with oxidative damage in rat's hippocampus. Pharmacol Biochem Behav. 102:196-202.
35. An L, Yang Z, and Zhang T. 2013. Melamine induced spatial cognitive deficits associated with impairments of hippocampal long-term depression and cholinergic system in Wistar rats. Neurobiol Learn Mem. 100:18-24.
36. Vanachayangkul P, and Tolleson WH. 2012. Inhibition of heme peroxidases by melamine. Enzyme Res. 2012:416062.
37. Xie G, Zheng X, Qi X, et al. 2010. Metabonomic evaluation of melamine-induced acute renal toxicity in rats. J Proteome Res. 9:125-33.
38. Bradley EL, Castle L, Day JS, et al. 2010. Comparison of the migration of melamine from melamine-formaldehyde plastics ('melaware') into various food simulants and foods themselves. Food Addit Contam A. 27:1755-64.
39. Chien CY, Wu CF, Liu CC, et al. 2011. High melamine migration in daily-use melamine-made tableware. J Hazard Mater. 188:350-6.
40. Wu CF, Hsieh TJ, Chen BH, et al. 2013. A crossover study of noodle soup consumption in melamine bowls and total melamine excretion in urine. JAMA Intern Med. 173:317-9.
41. Yang F, Mao Y, Zhang X, et al. 2009. LC-MS/MS method for the determination of melamine in rat plasma: toxicokinetic study in Sprague-Dawley rats. J Sep Sci. 32:2974-8.
42. Baynes RE, Smith G, Mason SE, et al. 2008. Pharmacokinetics of melamine in pigs following intravenous administration. Food Chem Toxicol. 46:1196-200.
43. Chan JY, Lau CM, Ting TL, et al. 2011. Gestational and lactational transfer of melamine following gavage administration of a single dose to rats. Food Chem Toxicol. 49:1544-8.
44. Xu S, Zhang Y, Sims A, et al. 2013. Fate and toxicity of melamine in activated sludge treatment systems after a long-term sludge adaptation. Water Res. online.