6 Melamine

Melamine is a trimer of cyanamide containing 66.6% in weight of nitrogen and it is component of the chemical family of triazine. The high content of nitrogen was the reason for implementing melamine in animal nutrition as a source of non proteic nitrogen (NPN). Different sources of NPN were tested between 1960s and '70s and also Artturi Ilmari Virtanen (Nobel Prize in Chemistry, 1945) spent the last part of his life studying the potentiality of NPN sources for ruminants nutrition, particularly urea (Virtanen, 1971). Apart from urea, researchers conducted also experiments to verify the efficacy and safety of several condensation products of urea in ruminants nutrition, such as urea-phosphate (Piva *et al.,* 1970; Maroadi *et al.,* 1972; Marranghelo *et al.,* 1975), biuret, triuret and cyanuric acid (Clark *et al.,* 1965; Piva *et al.,* 1970). Melamine is another condensation product of urea, and the lower efficiency respect to urea regarding the NPN source was tested by Newton & Utley (1978) in steers. Melamine was also proposed as a diuretic (Lipschitz & Stokey, 1945) but it was never used in pharmacology.

Melamine is not allowed as feed or food additive, but it is considered an indirect additive by FDA due to the fact it is highly diffuse in polymer resins and adhesives in food packages (List of Indirect Additives Used in Food Contact Substances, FDA, 2008). The migration of melamine in food ranges between 0.54 and 2.21 mg/kg acid matrix (e.g. coffee, lemon or orange juice) when the package is warmed at 95°C for at least 30 minutes (Ishiwata *et al.,* 1987). The estimated intake of melamine as indirect additive from food packages is 0.007 mg/kg body weight (OECD, 2002). According to the European Commission Decision 2008/921, 2.5 mg/kg is the maximum admitted level of melamine in food. Being the estimated migration of melamine from food packages quite low, values over the threshold in food is logically linked to a voluntary addition. The tolerable daily intake (TDI) of melamine is 0.5 mg/kg BW by EFSA (2008) and 0.63 mg/kg BW by FDA (2007).

Melamine intoxications: infant formula in China and pet-food in the USA

Between March and April 2007 there was a consistent recall of pet food in North America, following cases of illnesses in cats and dogs who had ingested certain lots of wet pet food. Even though the brands involved were numerous, the products were produced by a single manufacturing facility, with distribution contracts to many other pet-food companies. A joint effort between the involved companies and FDA performed an analysis of the event: in a first instance it was observed that pets suffered renal diseases up to renal failure or death. Secondly, the manufacturing facility was able to determine that the contaminated lots of pet food had been produced using a new supply of wheat gluten acquired from China. The symptoms of pets were anorexia, vomiting, lethargy, polyuria, and polydypsia; clinical analysis of blood samples showed high urea nitrogen and creatinine contents. The histopathologycal studies on dead animals showed the clear presence of renal failure due to yellowish-brown crystals (Dobson *et al.,* 2008).

Data from various laboratories in the United States agrees that the tainted matrix had a consistent amount of triazines (percentage of weight): 8.4% of melamine, 5.3% of cyanuric acid, 2.3 and 1.7% of ammelide and ammeline, respectively; and less than 1% of

both ureidomelamine and methylmelamine[1]. Apart from pet food, also swine, poultry and fish feed were tainted with triazine. FDA (2007) reported that lot of swine feeds were contaminated with 30-120, 5.6-10.8, 33.6-43.2, and 6.6-22.5, mg/kg of melamine, ammeline, ammelide and cyanuric acid, respectively. The investigated poultry feeds did not contain traces of melamine and ammeline, whereas had 2.11-2.63 mg/kg of cyanuric acid and 13.9 mg/kg of ammelide. The unique triazine in fish feeds was melamine (from 53 to 400 mg/kg). The analysis of tissues, from swine and poultry fed tainted feeds, reported no detectable melamine (the used Limit of Detection, LOD, was 50-100 µg/kg). On the basis of these data, FDA concluded that consumers high probably do not face health hazard when ingesting pork, chicken, eggs and domestic fish fed inadvertently contaminated diets. Particularly, the consumption of fish should not represent a health risk at melamine level lower than 50 µg /kg (FDA, 2007). However, Andersen *et al.* (2008) conducted a survey in North-American market-ready fish (shrimp, catfish, tilapia, salmon, eel, and other types, n = 105), showing that 9.5% of samples had melamine contamination higher than 50 µg /kg (ranging from 51 to 237 µg /kg), and 31.4% contained not detectable level of melamine (LOD was 3.2 µg /kg).

Following the feed contamination in North America, at the date of September 2008, almost 55 000 Chinese children suffered disease after ingestion of melamine tainted infant formula, about 13 000 were hospitalized and 4 of those died. The hospitalized children aged principally at most 3 years: 80% were under 2 y, and 17.33% between 2 and 3 years (Lam *et al.*, 2009). The clinical manifestation of involved children varied from asymptomatic urolithiasis to acute renal failure, hydronephrosis and death. A strong correlation between urinary melamine concentration and the formation of renal stones was demonstrated, and high probably infants and young children were more susceptible to toxic effects of melamine because of their immature metabolic and excretory mechanisms (Lam *et al.,* 2009). To make these effects worse was also the dose ingested by children in China. The infant formulas were contaminated with 2.5 g/kg of melamine, and WHO (2008) estimated that children ingested 8-25 mg melamine/kg BW, therefore doses from 16- to 50-fold higher than the European TDI of 0.5 mg/kg BW.

European cases of feed and food contamination with melamine

Following the EFSA opinion on melamine (EFSA, 2007), between September and December 2008, the European Commission issued three subsequent Decisions, (2008/757/EC; 2008/798/EC; and 2008/921/EC), for "imposing special conditions governing the import of products containing milk or milk products originating in or consigned from China". The first two decisions state that all composite products containing milk or milk products have to be systematically tested and the maximum allowed level of melamine is 2.5 mg/kg whole product. In the most recent decision, the safety controls are extended to ammonium bicarbonate for human and animal nutrition, feed and food containing milk, milk products, soya and soya products.

[1] These values are the approximate % by weight, determined for a single lot of tainted gluten. Contaminant concentration varied from lot to lot (based on comparison of results from FDA and other labs), with melamine usually present as the most abundant of the triazine contaminants in tainted materials. (*Original note to data from* Dobson *et al.,* 2008).

The brief updating between these three Decisions is a consequence of the EFSA statement on the risk factor of "protein rich-ingredients used for feed and food" (EFSA, 2007), and from the activity of the European Rapid Alert System for Food and Feed (RASFF)[2]. The latter, regarding melamine contamination, between March 2007 and April 2009 registered 33 alerts, 39 information notifications[3] and 15 rejections at the European custom, including five cases on ammonium bicarbonate (data source: RASFF Portal Database). About 90% of those contaminations originated from China, considering also the contaminated pet-food from the USA produced with tainted-gluten from China. In table 6 are summarized all cases of melamine contamination entered in the European market. Cereal, bakery products and confectionery were the principle contaminated human food, probably because of the presence of melamine in additives (e.g. gluten) and/or milk derivatives. The feed market was less involved than the human food, and the contamination regarded high protein feeds, such as rice protein concentrate, soybean and corn gluten. In October 2008, in Austria was also registered the presence of cyanuric acid (7.4 - 6.9 mg/kg) in sweet whey powder from Croatia. Feed cases, however, had the highest level of contamination in Europe (comparable to those of wheat gluten in the United States): in the United Kingdom it was found a rice protein concentrate with 36 g/kg of melamine, imported from China via Germany.

[2] The Rapid Alert System for Food and Feed (RASFF) is defined by the website www.eubusiness.com as "a quick and effective tool for the exchange of information between competent authorities when risks to human health are detected in the food and feed chain and measures -such as withholding, recalling, seizure or rejection of the products concerned- are taken. This quick exchange of information allows all members of the network to verify immediately whether they are also affected by the problem. Whenever the product is already on the market and should not be consumed, the authorities are then in a position to take all urgent measures, including giving direct information to the public, if necessary."

[3] The difference between Alert and Information notifications is given by the magnitude of risk effectively manifested in the market. Following the RASFF definitions:

→Alert notifications are sent when a food or feed presenting a serious risk is on the market and when immediate action is required. Alerts are triggered by the Member State that detects the problem and has initiated the relevant measures, such as withdrawal/recall. The notification aims at giving all the members of the network the information to verify whether the concerned product is on their market, so that they also can take the necessary measures

→Information notifications concern a food or feed that was placed on the market for which a risk has been identified, but for which the other members of the network do not have to take immediate action, because the product has not reached their market or is no longer present on their market or because the nature of the risk does not require any immediate action.

Table 6. Alert and Information notifications registered by the European Rapid Alert System for Food and Feed between March 2007 and April 2009. (Source: RASFF Portal Database, https://webgate.ec.europa.eu/rasff-window/portal)

	Alerts	**Informations**	***Total***
Human Food	**26**	**25**	***51***
Cereals and bakery products	11	10	
Confectionery	10	6	
Nuts, nut products and seeds	4		
Additives	1	2	
Dietetic foods, food supplements, fortified foods		1	
Milk and milk products		4	
Other food product		1	
Prepared dishes and snacks		1	
Feed	**5**	**11**	***16***
Pet food	**2**	**3**	***5***
Total	***33***	***39***	***72***

6.1 Chemical properties, synthesis and industrial applications

Melamine is component of the triazine family such as ammeline, ammelide and cyanuric acid (figure 11); all the triazines are linked by a hydrolytic pathway that from melamine produces cyanuric acid, carbon dioxide and ammine (see figure 12). Melamine and the other triazines differ between each other for the number of hydroxyl and/or ammine groups linked to the 1,3,5-triazine ring. One of the main characteristic of triazine is the possibility to create different kind of crystals due to the hydrogen bondings between hydroxyl and ammine, and the number of the latter is responsible for the chemical and physical properties of these crystals.

Melamine was firstly synthesized by the German chemist Justus von Liebig in 1834 by heating an intimate mixture of ammonium chloride and potassium thiocyanate. The modern process for producing melamine is based on the condensation of 6 moles of urea catalyzed by gas-phase or high pressure liquid-phase. If we take in consideration the gas-phase process, urea is initially transformed in cyanic acid and ammonia (endothermic step), then 6 molecules of cyanic acid are condensed forming melamine and carbon dioxide (exothermic step), for a final endothermic balance. The hot ammonia gas used to prevent the deammonization is then separated by the slurry of melamine. Once cooled and ammonia and carbon dioxide is eliminated, melamine is obtained as powder at 99.9% purity (Kirk-Othmer Encyclopaedia of Chemical Technology, 1978). Melamine was also produced from cyanamide and/or dicyandiamide in the simultaneous presence of alkali hydroxide. This industrial process (registered as United States Patent 4069383) is no more used, however being the calcium cyanamide a widely used fertilizer, the Fertilizer Institute performed a survey between calcium cyanamide producers and published a statement in which the president of Fertilizer Institute states that the fertilizer

manufacturers were not responsible for melamine-tainted milk (The fertilizer Institute, 2008).

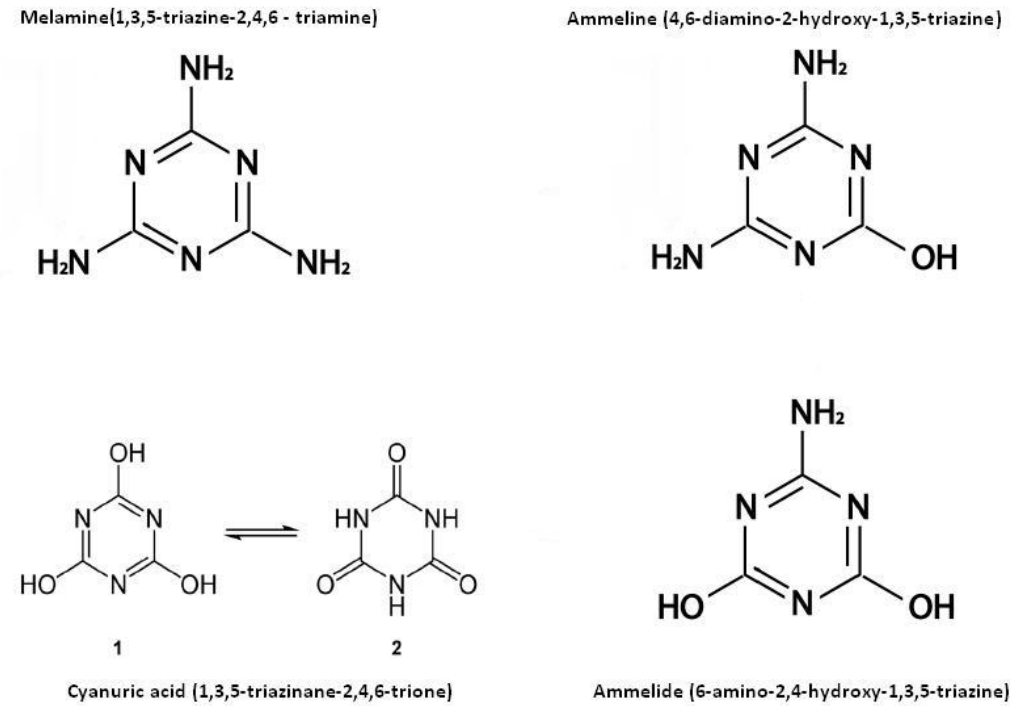

Figure 11. Chemical structure of triazines: melamine, ammeline, ammelide and cyanuric acid (within brackets the IUPAC name)

After its discovery, the melamine was commercially interesting only in the 1930s', since it was used in industrial applications including fabric impregnation, adhesives and moulding powders containing cellulosic fibers, fillers and pigments. Melamine and formaldehyde began start the *duroplast* innovation thanks to the thermosetting resin synthesized by their condensation. Nowadays, because of its excellent chemical stability, low fabrication cost and simple synthesis process, melamine is one of the most common molecular units contained in a huge number of daily objects. Moreover, the melamine is an excellent molecule for optical transmission; a very good flame retardant and an abrasion resistant; it is chemically inert and stable to heat, light, moisture and various chemicals attack. Therefore, between the industrial applications of melamine we can list: cross-linking additives for coatings of automotive and household parts; flame retardant additives for foams in furniture and mattresses; various moulded plastic wares; laminates for kitchen cabinets, floors and table tops; concrete plasticizers; resins for textile, paper finishing and others (Irimia-Vladu *et al.,* 2009).

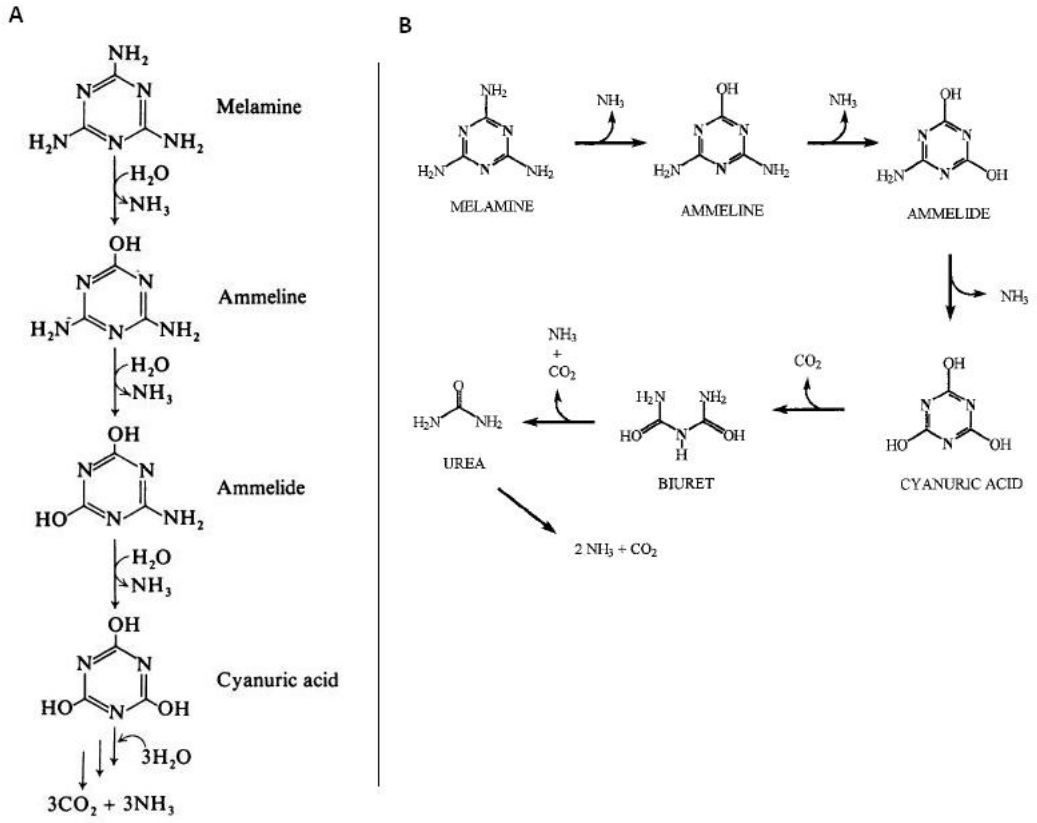

Figure 12. The hydrolytic metabolism of melamine for the formation of ammonia and carbon dioxide (part A), and the hypothetical complete metabolism (part B). Part A comes from a study on *Pseudomonas sp.* strain A (Jutzi *et al.,* 1982); Part B comes from the study on *Klebsiella terragena* (Shelton *et al.,* 1997), confirmed on *Enterobacter cloacae* and *Escherichia coli* by Cheng *et al.* (2005).

6.2 Metabolism of triazine family by bacteria

The hydrolytic reactions that can occur between triazines have been demonstrated by some authors. *Pseudomonas* sp. strain A was capable of growing using melamine, ammeline, ammelide, cyanuric acid or ammonia as sole and growth-limiting source of nitrogen (Jutzi *et al.,* 1982). The final growth yield of this microorganism was 50 and 45 g protein/mol of nitrogen when using ammonia and melamine, respectively. When melamine was used as nitrogen source, *Pseudomonas sp.* catalysed the hydrolytic pathway which stoichiometrically produced six moles of ammonia and three of carbon dioxide from a mole of melamine and six of water (figure 12 A). Another experiment using *Klebsiella terragena* and melamine as sole source of nitrogen (Shelton *et al.,* 1997), suggested that the transient accumulation of ammeline, ammelide and cyanuric acid in culture broth, could depend by the deamination of triazine ring. Therefore, a hydrolytic

metabolism including the production of biuret and urea (figure 12 B) was proposed by Shelton *et al.* (1997) and confirmed by Cheng *et al.* (2005). According to these authors, cyanuric acid would be decarbossilated to biuret plus a mole of water, then urea should be produced after decarbossilation and deamination of biuret. So, the final stoichiometric balance "melamine + 6 H_2O→ 6 NH_3 + 3 CO_2" remains unchanged.

6.3 Metabolism of melamine in mammals

After the melamine tainted-milk scandal there has been an increasing number of scientific publications on the health risk arising from the human ingestion of melamine: various studies on the clinical manifestations in human population or animals exposed to contamination, and animal models research about its toxicity. The data about metabolism of melamine are fragmentary and include mainly, the pharmacokinetic studies on pigs (Baynes *et al.,* 2008) and on rats by using a ^{14}C labelled melamine (Mast *et al.,* 1983).

According to Mast *et al.* (1983), when male Fischer rats were fed 1.3 mg melamine/kg BW within the first 24 hours about 90% of the ingested melamine was excreted via urine. After 96 h from ingestion, the total excretion was 93±4% in urine and only 0.2% and 0.6% in breath and faeces, respectively (figure 13A). In a further analysis the content of melamine in faeces was probably given by the contamination with urine in the cage; the authors found an additional 4% in the cage washing, which probably was excreted via urine in the first 24 h. Then, considering all the excretion routes, the total elimination of melamine after 96 h the ingestion was about 99%. The kinetic analysis in rat showed that the half-life elimination of melamine was 3 hours, with a renal clearance of 2.5±0.1 ml/min.

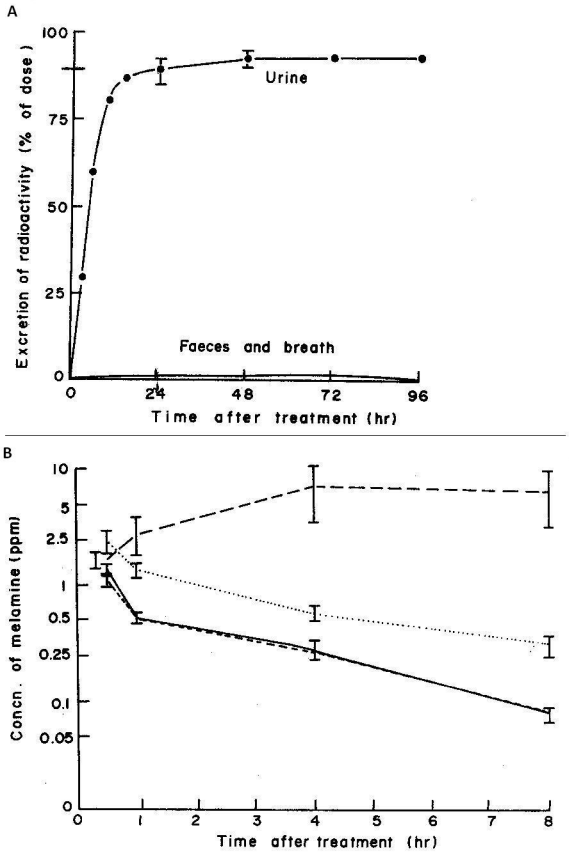

Figure 13. Kinetic graph on the urinary elimination of melamine (part A), and on the distribution in tissues of male Fischer rat (part B). Liver (—); kidney (---); bladder(– –); plasma (···). From Mast *et al.,* (1983)

The distribution of melamine in plasma, liver, kidney and bladder is shown in figure 13B. After 30 minutes the ingestion, concentration of melamine was maxima in plasma, liver and kidney, and minima in bladder. After 24 h the content of melamine (ng/g) was zero in blood and plasma, 1.3±0.7 in kidney, 1.8±0.1 in liver, 12±4 in urethra, and 31±15 in

bladder. At 96 hours the only significant amount of melamine was found in bladder (8±2) and urethra (13±5). According to Mast *et al.* (1983), therefore, melamine was not metabolized by male Fischer rats.

The most recent study by Baynes *et al.* (2008) was to clarify the distribution and excretion of melamine avoiding the confounding factors associated with the oral absorption or other extravascular routes of administration. The volume distribution of melamine in pigs was 0.61±0.04 L/kg, and being similar to total body water it was highly probable that the molecule was distributed mainly in the extracellular fluid compartment. By being a base, the melamine should be absorbed in the small intestine (Dobson *et al.,* 2008) and according to Cianciolo *et al.* (2008), because of its high polarity, it is unlikely that melamine can be stored in adipose tissue and released over time. This characteristic was also confirmed by the chemical properties of melamine, which has pK_b of 9.0 and molecular weight of 126.12, which means that is a polar and small amine. Smith *et al.* (1994) demonstrated that this kind of molecules follows the previous described pharmacokinetic behaviour. In pigs the volume distribution was about 3-fold lower than rat (1.8 L/kg, Mast *et al.,* 1983), suggesting their kidney cleared more rapidly than those of rat. Baynes *et al.* (2008) concluded that pig's blood would be cleared of about 99% of ingested melamine via urine within 28 h after assumption, and when pigs are fed 6.13 mg melamine/kg DM, the related content in blood should be below the safe level of 50 µg/ml proposed by the US Food Safety Inspection Service (USDA, 2007).

These study on metabolism of melamine after being ingested at doses closely lower (0.5 mg/kg DM, Mast *et al.,* 1983) or higher (6.13 mg/kg DM, Baynes *et al.,* 2008) than the maximum permitted level in Europe (2.5 mg/kg DM) showed a rapid and almost complete clearance of melamine by kidney. According to the data on the rapid clearance, at safe ingestion level, the melamine and its analogues does not accumulate in mammalian tissues (EFSA, 2007). However, further studies in other species could be useful to better determine the depletion kinetics of melamine, ingested at level close to the maximum level allowed in food.

6.4 Acute toxicity on renal system and carcinogenety

Melamine has as similar acute toxicity as sodium chloride (LD_{50} of NaCl is 3 g/kg), with a LD_{50} of 3.16 g/kg in rodents; the Threshold Limit Value (TLV) of melamine in the environments is 10 mg/m^3 (OECD, 2002). According to the study on rats by the International Agency for Research on Cancer (IARC, 1999), the main toxic effects after repeated melamine ingestion are calculi formation, inflammatory reactions and hyperplasia in the urinary bladder. The negative effects of melamine on charge of the renal system appeared to be sex and specie-specific. In toxicological experiment lasting 90 days, the formation of urinary bladder stones occurred in male rats ingesting 1.5 g/kg of melamine (150 mg/kg BW), while no stones formation was observed in female rats ingesting up to 12 g melamine/kg per day (NTP, 1983).

Recently, it was supposed that dermatitis caused by melamine-formaldehyde resins could be addressed to the effect of melamine instead of formaldehyde (Aalto-Korte *et al.,* 2003).

Melamine is not considered carcinogenic, genotoxic or teratogenic, however “there is sufficient evidence in experimental animals for the carcinogenicity of melamine under conditions in which it produces bladder calculi” (IARC, 1999). More recently Cohen (in Meek *et al.,* 2003) states that the urinary tract calculi increased the incidence of bladder tumors in rodents by a non-DNA-reactive mode of action involving ulceration and regenerative hyperplasia. The Scientific Committee of Food (European Commission, 1986) fixed firstly in Europe the TDI of melamine to 0.5 mg/kg BW (then confirmed by EFSA in 2008). The American TDI of 0.63 mg/kg BW was instead chosen by applying a safety factor of 100 to the NOAEL (No Observable Adverse Effect Level) of 63 mg/kg BW (FDA, 2007).

In human, according to Lam *et al.* (2009) the urine melamine/creatinine ratio (y in µg/mmol) is dependent by the diameter of melamine-induced stone (x in mm) as follows: $y = 10.3x - 24.0$ ($r^2 = 0.81$, $P<0.001$). In the Chinese children exposed to tainted infant formula, the formation of stones could also be affected by hyperuraturia, since melamine associated renal stones contained also uric acid (Sun *et al.,* 2008).

Considering also cyanuric acid, although both molecules have low acute toxicity and are not considered as direct carginogenic, genotoxic and teratogenic molecules, following the contamination of pet-food with a mixture of triazine, it was also established a combined toxicity of melamine and cyanuric acid (Dobson *et al.,* 2008; Cianciolo *et al.,* 2008; Reimschuessel *et al.,* 2008)

6.4.1 Melamine and melamine-cyanuric acid toxicity

The effect of melamine (or triazine mixture) ingestion in pets and children were highly variable according to the level of contamination, to the age and to the individual difference between subjects (Cianciolo *et al.,* 2008; Lam *et al.,* 2009). Two interesting experiments about the effect of melamine, either melamine and cyanuric acid, or triazine mixture ingestion were performed by Reimschuessel *et al.* (2008) in pigs and fish, and in rats by Dobson *et al.* (2008).

Just before the FDA recall for melamine contamination in pet food, Cianciolo *et al.* (2008) inadvertently exposed different experimental groups of cats (total of 70 subjects) to melamine and cyanuric acid contaminated pet food for 4 and 6 days. The content of melamine and cyanuric acid in those feeds ranged from 1.6 to 2.2, and from 0.32 to 0.59 g/kg, respectively. When rats and dogs ingested this level of cyanuric acid no adverse effects were observed (Canelli, 1974), neither does exist, to our knowledge any documented case of severe renal disease after ingestion of cyanuric acid alone. In Cianciolo *et al.* (2008), 43 cats showed the following clinical signs: inappetence, vomiting, polyuria, polydipsia and lethargy. One cat died and 13 were euthanized. The histologic examination of dead cats revealed the formation of intratubular crystalluria (figure 14), tubular necrosis and inflammation states (the list of histologic signs has been simplified).

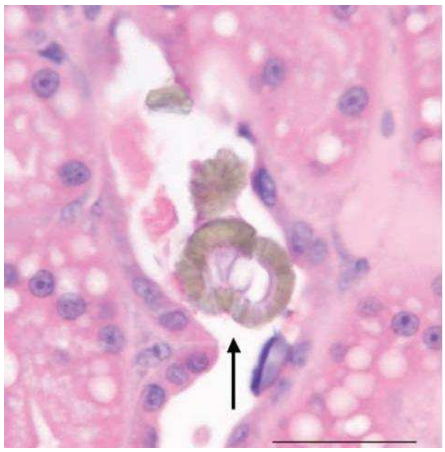

Figure 14. Photomicrograph of a section of kidney from a cat euthanized approximately 2 weeks after eating pet food contaminated with melamine and cyanuric acid. The arrow indicates a gold-brown circular crystal (20-30 µm in diameter) in a renal tubule. Source: Cianciolo *et al.* (2008)

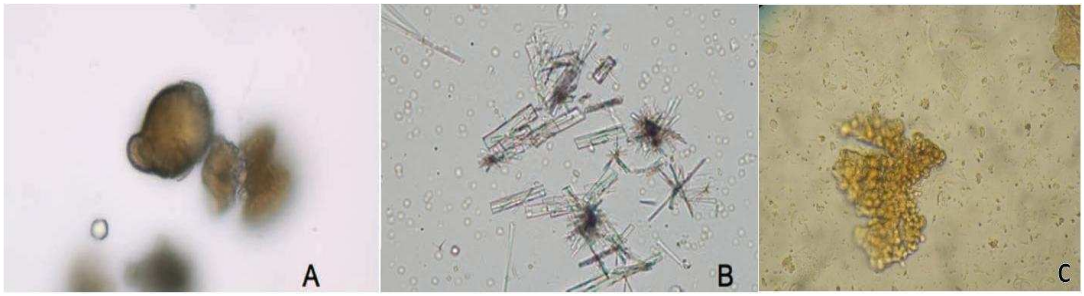

Figure 15. Photomicrographs of circular green-brown crystals in urine from a cat that ingested food contaminated with melamine and cyanuric acid (A); irregular needle-shaped crystals obtained by adding melamine to urine from a cat (B); melamine-associated crystals in a children ingested tainted milk (C). Source: A-B from Cianciolo *et al.* (2008), C from Lam *et al.* (2009).

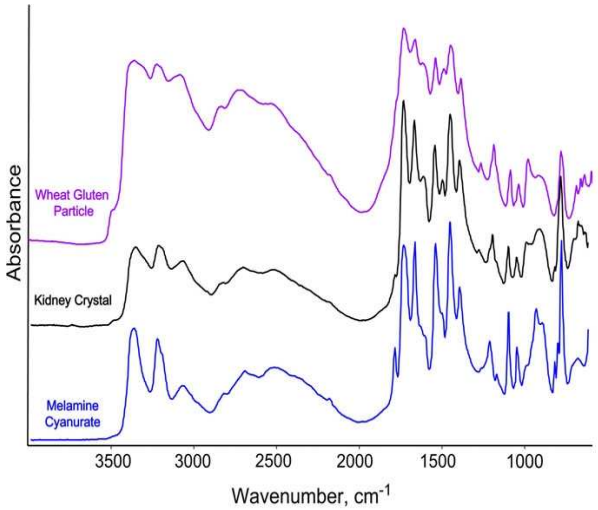

Figure 16. Comparison of Fourier Transformed Infrared (FTIR) spectroscopy from a particle isolated from a suspect wheat gluten (top), a crystal located within a cat ingested contaminated gluten (middle), and a melamine-cyanuric acid (1:1) cocrystal reference compound (bottom). Source: Dobson *et al.* (2008)

Figure 15A shows that the melamine-cyanuric acid tubular stones (from now MACC) appear yellowish-brown crystals, i.e. completely different to the white needle crystals formed in the urine by melamine (figure 15B). MACC, moreover, is more consistent than the friable melamine stones revealed in exposed Chinese children (figure 15C). Dobson *et al.* (2008) clarified that MACCs were the principle responsible of the American pets disease. The authors compared by Fourier Transform Infrared spectroscopy (FTIR) the chemical structure of:

1) Contaminated wheat gluten, suspected to be responsible for pets diseases
2) Section of kidney tissue (5 μm) from cats and rats fed the contaminated and the experimentally contaminated feed, respectively
3) Melamine-cyanuric acid cocrystal reference material (ratio 1:1 in weight)

The results (figure 16) provide a definitive analytical proof that MACCs were contemporary contained in the contaminated diets and in the tubular kidney of involved pets. MACC is insoluble in water and has a lattice structure formed by alternating units of melamine and cyanuric acid. MACC, particularly appeared stable during feed mill processing and storage. It was observed on rats that MACC was ingested as whole and then dissociated at low pH in the gastric lumen, then cyanurate should be absorbed by the stomach, and melamine by the small intestine. The pathway of melamine and cyanuric acid appeared similar and authors were inconclusive about the reason why MACC was formed back only in kidney tubules. An explanation could be that melamine and cyanuric acid concentrations need to exceed a critical point, which might occur as they progress down to the osmotic gradient in kidney (Dobson *et al.,* 2008).

The chemical property of MACC and other less consistent crystal formed with different triazines can be fundamental to better understand the toxic effect on the renal system about these compounds once ingested with the diet. For instance, Sun *et al.* (2008) found uric acid in renal stones of children ingesting tainted milk with melamine; ureidomelamine (urea plus melamine) increased in kidney tissues of cats and rats, respectively, accidentally and experimentally exposed to mixture of triazine (Dobson *et al.,* 2008). Besides, Reimschuessel *et al.* (2008) considered the role of the melamine-cyanurate complex on urate oxidase (converting uric acid to allantoin but not present in humans) in animals as possible cofactor in the pathogenesis of renal failures.

An experimental study in fish and pigs fed 400 mg/kg of melamine and cyanuric acid alone or a mixture containing 400 mg/kg of each molecule confirmed the single low toxicity and the occurrence of renal failure following their simultaneous ingestion. In fish, the ingestion of only melamine or cyanuric acid resulted in a higher concentration of these in the edible tissues, probable by the lack of crystal precipitation in the gastrointestinal tract and kidney when fed together (Reimschuessel *et al.,* 2008).

6.5 Carry over of melamine from feed to milk

Studies on ruminants to verify the toxic effect of melamine and its role as NPN in ruminants were performed in 1966 and 1978, but no info were given on possible transfer from feed to milk (from now excretion pattern). Sheep weighing 35 kg had no adverse effect when fed 7 g/d of melamine, whereas they incurred in crystalluria and consequent death when the diet contained more than 10 g melamine per day (Clark, 1966). Newton &

Utley (1978) reported that melamine could not provide adequate levels of ammonia for maximum microbial protein synthesis, in their experimental condition. Therefore, considering also the well defined hydrolytic metabolism of melamine by bacteria, a certain hydrolysis of melamine in the rumen could take place.

To our knowledge only Cruywagen *et al*. (2009) measured the excretion patter (EP) of melamine in milk in lactating dairy cows. The authors, simulating a lot of contaminated corn gluten, fed 17.1 g/day/head of melamine to cows, for 8 consecutive days. The concentration of melamine in milk (figure 17) reached its maximum level (about 15 mg/kg) at day 3 after the beginning of treatment and rapidly decreased after the melamine withdrawal. The total disappearance of melamine in milk occurred after 152 h the last administration. The mean EP during the treatment period ranged between 1.7 to 2.1%. The authors reported no effect of feeding melamine on daily milk production, milk fats, proteins, lactose and urea. Considering data of Newton & Utley and the hydrolysis of melamine, it is reasonable to think that melamine could affect the concentration of urea in milk (Cruywagen *et al.,* 2009).

It is clear that high contamination of melamine in livestock feed can pose a serious health risk for population drinking milk. In particular, it should be considered the enrichment factor in powder milk used for infant formula and other human foods. The work of Cruywagen *et al.* (2009), however, having not considered other doses of melamine, above all close or similar to the maximum permitted concentration of melamine in food and feed settled by Europe (2.5 mg/kg), cannot help us to predict a risk arising when lactating cows are fed low contaminated feeds.

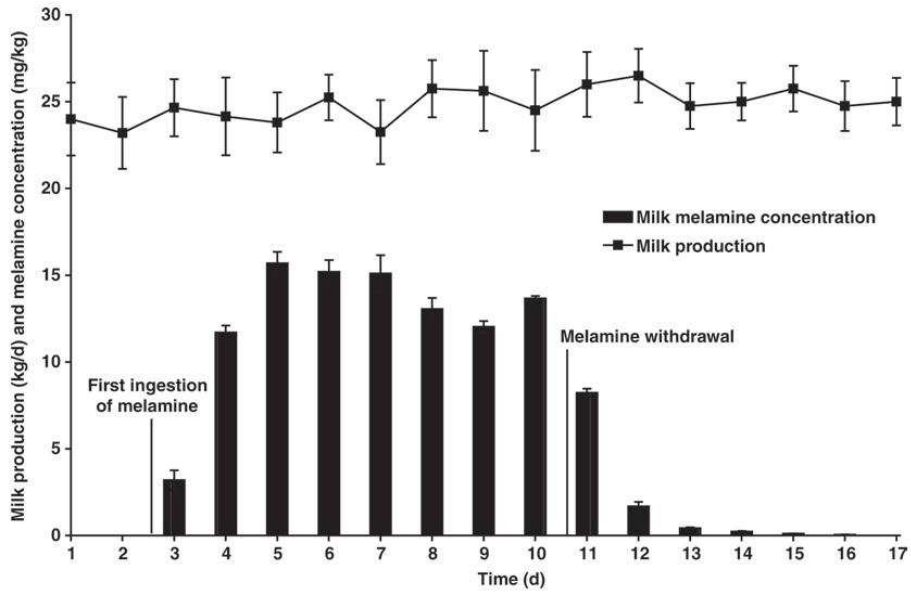

Figure 17. Milk production and melamine concentration of milk from cows that ingested 17.1 g/day of melamine. Source: Cruywagen *et al.* (2009).