Chapter I

Introduction

1.1 Melamine

Melamine (2,4,6-triamino-1,3,5-triazine) is produced in high volumes mainly for the synthesis of melamine–formaldehyde resins, one of the uses of which includes the manufacture of contact materials such as plastics, laminates and coatings. Additionally, melamine is present as a trace contaminant in nitrogen supplements used in animal feeds (e.g. urea), and can be found as a metabolite and degradation product of the pesticide and the veterinary drug, cyromazine (EFSA, 2010).

Contamination of food and feeds can occur through several different routes including: 1) trace levels of melamine found in the environment contaminating the food chain; 2) the accidental contamination of animal feed or human food that have been treated with products that contain melamine, such as fertilizer and pesticides; 3) the leaching of melamine monomers from plastic and tableware products; 4) or adulteration which is “the intentional addition of melamine or its analogues directly to food, food ingredients, animal feed, feed ingredients, or pelletizing agents” (Hilts and Pelletier, 2009). The addition of melamine, which contains 66% nitrogen by mass, to food to falsely elevate results of protein content assays has recently become a serious concern (Brown *et al.*, 2007 and Puschner *et al.*, 2007).

Baseline concentrations of melamine are present in the environment and in the food chain as a result of the widespread use of materials that contain melamine. Generally, baseline levels are expected to be < 1 mg/kg (WHO, 2009a).

The World Health Organization (WHO) also published guidelines regarding melamine in 2008, where information about melamine and the food recall is outlined. United States Food and Drug Administration (U.S. FDA's) interim safety/risk assessment on melamine and its related triazine compounds set the tolerable daily intake (TDI) at 0.63 mg/kg B.W/day (FDA, 2009).

The toxicity of melamine alone is very low, and greater than 90% of the ingested melamine is eliminated within 24 h in an animal experiment, although melamine toxicity is low, most animal studies showed effects on health following sub-acute or chronic melamine exposure (Brown *et al.*, 2007 and Baynes *et al.*, 2008).

Crude melamine may contain several by-products (ammeline, ammelide and cyanuric acid), thus these triazine-based chemicals may also be present in contaminated foods alongside melamine. Although these melamine-related compounds are considered relatively less toxic when administered individually, melamine, together with its by-products (cyanuric acid) have been linked to acute renal failure and other health problems because of the formation of insoluble melamine-cyanurate crystals in the kidney. Unfortunately, the illegal use of melamine as a food ingredient has led to many poisoning incidents of cats and dogs in the United States, as well as renal function failure of Chinese infants (Heller and Nochetto, 2008 and Klampfl *et al.*, 2009). Fluid therapy has been effective in restoring health in animals developing renal failure (Burns, 2007a).

The most common methods used to detect melamine in foods and feedstuff are gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) combined with ultraviolet (UV) or mass spectrometry (MS) detectors (Tyan *et al.*, 2009).

1.2 Honey

Honey, a sweet food made by bees using nectar from flowers has been proven to be of medicinal importance both at curative and preventive levels. It is a promising antitumor agent with pronounced antimetastatic and antiangiogenic effects (Hanaa and Shaymaa, 2011), antiviral, antiinflammatory (Al-Waili, 2003 a), pain reducing (Azimi *et al.,* 2007), immunostimulatory, prebiotic (Chick *et al.,* 2001).

Honey is a natural antioxidant which may contain flavinoids, ascorbic acid, tocopherols, catalase, and phenolic compounds all of which work together to provide a synergistic antioxidant effect, scavenging and eliminating free radicals (Johnston *et al.,* 2005). Its protective role against the kidney dysfunctions induced by sodium nitrite, a known food additives, hepatoprotective, hypoglycemic, reproductive, antihypertensive and of course antioxidant effects has also been reported (Hassan, 2007 and Omotayo *et al.*, 2012).

1.3 Aim of work

The objective of this study was to evaluate the role of natural bees honey against the adverse effects on the kidney functions induced by melamine feeding in male rats.

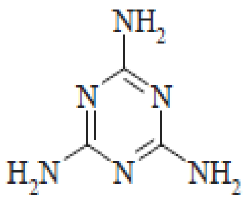
Chapter II

Review of Literature

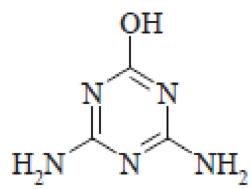
2.1 Chemical, biological and toxicological aspects

2.1.1 Chemistry and structure of melamine

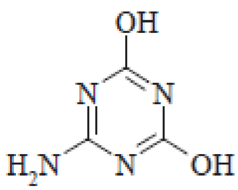
Melamine is a nitrogen heterocyclic triazine compound. It is commonly referred as triamines or protein essence and also known as 2,4,6-triamino-1,3,5-triazine, 1,3,5-triazine-2,4,6-triamine, 2,4,6-triamino-s-triazine, melamine amide and cyanuric triamide (Figure 2.1) (Tyan *et al.,* 2009).



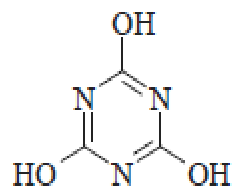
Melamine



Ammeline



Ammelide



Cyanuric acid

Figure 2.1: Structures of melamine and melamine structural analogs (Puschner *et al.,* 2007).

Melamine is a white, crystalline powder (Table 2.1), with a molecular formula of $C_3H_6N_6$. It is a small polar molecule, nonflammable, incompatible with strong acids or oxidizing agents and has several basic amino groups (Baynes *et al.*, 2008).

Table 2.1: Summary of the chemical properties of melamine (Hau *et al.,* 2009).

Property	Description
Appearance at room temperature	White 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
	Insoluble in diethyl ether
Melting point	357°C
Specific gravity	1.573 g/cm³
Molecular weight	126.12 g/mol

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 and Bizzari and 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 and Yokose, 2008). Applications of melamine include use in produce plastics, laminates, glues, resins, adhesives, tableware, coatings, leather, paints, flame retardants and fertilizers (Osborne *et al.*, 2008).

Additionally, cyromazine is an example of a chemical insecticide that contains melamine and acts as an insect growth regulator, though it is a highly effective pesticide, it can potentially be degraded to melamine (Chou *et al.*, 2003). Melamine derivatives of arsenical drugs are potentially important in the treatment of African trypanosomiasis (Barrett and Gilbert, 2006).

The use of melamine as non-protein nitrogen (NPN) for cattle was described in (Colby and Mesler, 1958, patent). Melamine "may not be an acceptable non-protein nitrogen source for ruminants" because its hydrolysis in cattle is slower and less complete than other nitrogen sources such as cottonseed meal and urea (Newton and Utley, 1978).

Melamine is sometimes illegally added to food products in order to increase the apparent protein content. Standard tests such as the Kjeldahl and Dumas tests estimate protein levels by measuring the nitrogen content, so they can be misled by adding nitrogen-rich compounds such as melamine (Moore *et al.*, 2010).

Melamine was found in liquid milk, yogurts, frozen desserts, powdered milk, cereal products, confectionaries, cakes, biscuits, protein powders, and some processed foodstuffs. Subsequently, a variety of nondairy products originating from China were found to be contaminated with melamine. These products included ammonium bicarbonate, animal feed and animal feed ingredients (RASFF, 2008). The maximum residue level (MRL) for melamine set by many countries and by WHO are 1 mg $kg^{-1}$for infant formula and 2.5 mg kg^{-1} for other milk and milk-based foods or all other foods (FAO/WHO, 2010).

Tolerable daily intake (TDI) of melamine established by the U.S/FDA in 2007 is 0.63 mg/kg per B.W for adults and 0.32 mg/kg per B.W for children (US FDA, 2007).

However, during the manufacturing process, several other compounds (ammeline, ammelide, and cyanuric acid) may be produced as co-contaminants, and their combination with melamine is toxic (Filigenzi *et al.*, 2008). These class of chemicals known as s-triazines (Wackett *et al.*, 2002). Hydrolysis or amination of one s-triazine can result in the production of another s-triazine (Baynes and Riviere, 2010).

Cyanuric acid is also a triazine (1,3,5-triazine-2,4,6-trihydroxy) (OSHA, 2010). However, used in swimming pools as a stabilizer and protects chlorine from being degraded by the sun's ultraviolet rays (Cantú *et al.,* 2001). The WHO also established a TDI for cyanuric acid at 1.54 mg/kg B.W (WHO, 2007).

Melamine and cyanuric acid are able to form self-assembling, high molecular weight complexes called melamine–cyanurate complex (Figure 2.2) via hydrogen bonds (Seto and Whitesides, 1993). This complex is practically insoluble in water. Reimschuessel also hypothesized that uric acid possessing imide structure might have the ability to interact with melamine to form melamine–urate complex via hydrogen bond networks (WHO, 2009b).