

**Melamine and Cyanuric acid:
Toxicity, Preliminary Risk Assessment and Guidance on Levels in Food
25 September 2008**

Introduction	1
Uses and possible human exposure	2
Toxicity of Melamine	2
Toxicity of cyanuric acid	3
Combined toxicity	4
Safety/Risk Assessment	4
Guidance on levels of health concern in foods	5
References	6

Introduction

An increased incidence of kidney stones and renal failure in infants has been reported in China, believed to be associated with the ingestion of infant formula contaminated with melamine. It has been discovered that melamine has been deliberately added to raw milk for a number of months in order to boost its apparent protein content.

In 2007 there was a large outbreak of renal failure in cats and dogs in the USA associated with ingestion of pet food found to contain melamine and cyanuric acid. The melamine was added deliberately to one of the ingredients. Melamine alone is of low toxicity, however experimental studies have shown that combination with cyanuric acid leads to crystal formation and subsequent kidney toxicity. It is not known whether the cyanuric acid was also added deliberately or whether it was a by-product of the melamine preparation added. Analysis of the contaminated ingredient (gluten) responsible for this outbreak revealed the following triazine compounds: melamine 8.4%, cyanuric acid 5.3%, ammelide 2.3%, ammeline 1.7%, ureidomelamine and methylmelamine both <1% (Dobson et al 2008).

It appears that melamine can be found in a variety of milk and milk products at varying levels, from low ppb to ppm ranges. Following the pet food incident in 2007 preliminary risk assessments have been published by nationals/regional authorities. These form the basis for this preliminary guidance developed to assist in the decision-making process on possible health concern of melamine levels in food.

This preliminary guidance is proposed as a first pragmatic approach until more data become available that allow a more detailed assessment. It has to be noted that currently there are large uncertainties involved in the assessment which preclude a more detailed guidance and emphasize the need for more data. WHO is presently initiating action to develop a more thorough assessment through meetings of international scientists.

Uses and possible human exposure

Melamine (CAS No. 108-78-1) is used in the production of melamine resins, typically by reaction with formaldehyde. It has many industrial uses, including in the production of laminates, glues, adhesives, moulding compounds, coatings and flame retardants.

In the US melamine is an indirect food additive for use only as a component of adhesives. [21 CFR 175.105; U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from: <http://www.gpoaccess.gov/ecfr> as of June 18, 2007]

Melamine was also found as a metabolite of the pesticide cyromazine in plants, goats, hens and rats (JMPR Report 2006). Melamine is also used in some fertilizers.

Outside the current food safety incident consumer exposure to melamine is considered to be low. Besides low levels of residues as consequence of cyromazine metabolism it may occur through the extraction of melamine from compression moulds by acidic foods, such as lemon or orange juice or curdled milk, at high temperature. Taking into account these sources the estimated oral uptake of melamine is around 0.007 mg melamine/kg body weight/day (OECD 1998).

Cyanuric acid (CAS No 108-80-5) is a structural analogue of melamine. It may be found as an impurity of melamine. Cyanuric acid is an FDA-accepted component of feed-grade biuret, a ruminant feed additive. It is also found in swimming pool water as the dissociation product of dichloroisocyanurates used for water disinfection. Consumer exposure may be through swallowing swimming pool water, through drinking water processed from surface water, and through fish which may accumulate this chemical (OECD 1999). When used in drinking water for disinfection purposes, sodium dichloroisocyanurate is rapidly dechlorinated to cyanurate.

Due to the widespread use of melamine, also in material in contact with food, low levels may be detected in food, not necessarily due to adulteration. Some countries have established legal limits in relation to migration of melamine from food contact material into foods.

Toxicity of Melamine

Melamine is not metabolized and is rapidly eliminated in the urine with a half life in plasma of around 3 hours (OECD 1998). The compound has a low acute toxicity, with an oral LD₅₀ in the rat of 3161 mg/kg body weight (OECD 1998).

No human data could be found on the oral toxicity of melamine. Data are available from animal feeding studies carried out in rats, mice and dogs. The main toxic effects of dietary exposure to melamine in rats and mice were calculi formation, inflammatory reactions and hyperplasia in the urinary bladder (OECD 1998, Melnick et al 1984; Bingham et al 2001; IARC 1986). Melamine crystalluria has been reported in dogs (Bingham et al 2001).

Haematuria has been reported in rats (IUCLID 2000). The lowest no-observed-effect-level (NOEL) for bladder calculi is 63 mg/kg/day in a 13 week rat study (OECD 1998).

Rodent studies have shown a differentiation of effects between males and females, with males more likely to develop bladder calculi (DHSS/NTP). There are also species differences in the incidence of bladder calculi, believed to be due to toxicokinetic differences.

Analysis of the bladder calculi has shown that these are constituted by melamine and uric acid, or melamine in a matrix of protein, uric acid and phosphate (Ogasawara H et al 1995; OECD 1999).

Renal toxicity of melamine

Most of the sub-chronic and chronic feeding studies in animals have failed to show any renal toxicity. In female rats, however, a 13 week feeding study found dose-related calcareous deposits in the proximal tubules, and following a 2 year feeding study chronic inflammation of the kidney was seen (DHSS/NTP).

In rats and dogs, high doses of melamine had diuretic properties, but did not produce renal toxicity (Lipschitz and Stokey, 1945).

Carcinogenicity

Induction of carcinomas of the urinary bladder occurred in male rats fed diets containing melamine at 4500 ppm (equivalent to 225 mg/kg bw per day) for 103 weeks, but not in female rats or in male or female mice (JMPR 2006). Tumours are significantly associated with the development of bladder calculi (DHSS/NTP 1983) and are related to the administration of high doses.

Melamine is not genotoxic in vitro or in vivo.

The WHO International Agency for Research on Cancer has concluded that there is sufficient evidence in experimental animals for the carcinogenicity of melamine under conditions in which it produces bladder calculi. There is inadequate evidence for carcinogenicity in humans (IARC 1999).

Toxicity of cyanuric acid

Cyanuric acid has low acute toxicity in mammals, with a rat oral LD₅₀ of 7,700 mg/kg body weight for rats (OECD 1999). Several subchronic oral toxicity studies have demonstrated that it causes renal tissue damage, including dilatation of the renal tubules, necrosis or hyperplasia of the tubular epithelium, increased basophilic tubules, neutrophilic infiltration, mineralization and fibrosis. These changes were probably caused by cyanurate

crystals in the renal tubules (OECD 1999). The no-observed-adverse-effect-level (NOAEL) for these effects is 150 mg/kg/day (OECD 1999).

In humans, more than 98% of an orally administered dose of cyanuric acid is excreted unchanged in urine within 24 hours (Allen et al 1982).

Sodium cyanurate was tested in several short- and long-terms studies in rats and mice. It did not induce any genotoxic, carcinogenic or teratogenic effect. Effects observed in rats and mice at high doses were occurrence of bladder calculi, epithelial hyperplasia of the bladder and at longer-term studies also renal tubular nephrosis. The NOAEL for sodium cyanurate derived from the 2-year study in rats was 154 mg/kg b.w. per day (WHO 2004).

Combined toxicity

While melamine and cyanuric acid are of low acute toxicity, evidence from the outbreak of acute renal failure in cats and dogs in 2007 associated with the consumption of contaminated pet food suggests that when melamine and cyanuric acid are co-ingested renal toxicity results. In the pet food incident, analysis of the food revealed the presence of a number of triazine compounds in the feed including melamine and cyanuric acid. A small study in which cats were fed increasing amounts of melamine and cyanuric acid also reported renal failure and the presence of renal crystals (Brown et al., 2007; Puschner et al., 2007). This was confirmed by Dobson et al (2008) who conducted a rat study that tested ingestion of melamine alone, ammeline or ammelide alone (both analogs of melamine), a mixture of melamine and cyanuric acid and a mixture of all four compounds. Neither ammeline nor ammelide alone produced any renal effects, but the mixtures produced significant renal damage and crystals in nephrons. Analysis confirmed the presence of melamine and cyanuric acid in the kidney. Infrared microspectroscopy on individual crystals from rat and cat (from the pet food outbreak) kidneys confirmed that they were melamine-cyanuric acid co-crystals.

Melamine cyanurate has very low solubility and it is hypothesized that this leads to the formation of melamine cyanurate crystals in the kidney. It is assumed that melamine and cyanuric acid are absorbed in the GI tract, distributed systemically and, for reasons that have not yet been fully determined, precipitate in the renal tubules leading to progressive tubular blockage and degeneration (Dobson et al, 2008).

Safety/Risk Assessment

Following above mentioned pet food incident and possible contamination of animal feed, the US FDA and the European Food Safety Agency (EFSA) performed preliminary risk assessments in 2007.

The US FDA has published an interim safety/risk assessment on melamine and structural analogues and has established for melamine a tolerable daily intake (TDI) of 0.63 mg per kg of body weight per day.

<http://www.cfsan.fda.gov/~dms/melamra.html>

EFSA has published a provisional statement and recommended to apply a TDI of 0.5 mg per kg of body weight per day for the total of melamine and its analogues (ammeline, ammelide, cyanuric acid).

http://www.efsa.eu.int/EFSA/Statement/efsa_statement_melamine_en_rev1.pdf?ssbinary=true

An updated Statement of EFSA on risks for public health due to the presences of melamine in infant milk and other milk products in China has been issued on 24th of September.

http://www.efsa.eu.int/EFSA/efsa_locale-1178620753812_1211902098495.htm

These values are based on experimental toxicity studies with melamine. Since there are no studies available investigating the combined toxicity of melamine and cyanuric acid together that would allow derivation of a tolerable intake value, it is recommended to use the currently proposed TDI values.

Guidance on levels of health concern in foods

The US FDA has published in their interim assessment an approach on how to determine a 'level of concern' for specific food products. This is the level at which the TDI would be reached when taking local consumption patterns and target population into account.

This approach can be applied to get an indication of what levels of contamination of melamine would raise potential health concern in specific foods.

In this approach one has to consider the average body weight of the target population to calculate the tolerable amount of melamine per person per day, then consider the amount of respective food eaten per day.

Considering a TDI of 0.5 mg/kg body weight, this would lead for a 50kg person to a tolerable amount of 25 mg melamine per day. Assuming this person would drink one litre of milk per day, this would indicate that the TDI would be reached at a level of 25 mg melamine per litre of milk. This level then would be considered as the 'level of concern'.

Considering a 5kg infant, the tolerable amount of melamine would be 2.5 mg per day. This amount would be reached when consuming 750 ml liquid (or reconstituted) formula contaminated at a level around 3.3 mg/l (ppm).

For comparison, the Sanlu product incriminated in the cases in China was contaminated at a level of over 2500 mg/kg powder, corresponding to approximately 350 ppm in reconstituted product (assuming a 7-fold reconstitution factor).

It has to be noted that this approach has a large uncertainty. For melamine toxicity species differences in toxicokinetics have been reported and insufficient information is available regarding specific sensitivity of infants. There is also a lack of information on the interaction of melamine and cyanuric acid. Also, dietary exposure to melamine and structural analogues as result of migration from food contact material or from other sources is not included, but this source of exposure can normally be considered low.

Therefore, when considering regulatory actions to be taken on contaminated food products potential human health impact, availability of foods and other factors should be taken into account. Investigations into source(s) of melamine contamination should be carried out in all cases to the extent possible.

References

- Allen LM, Briggles TV, Pfaffenberger CD (1982). Absorption and excretion of cyanuric acid in long-distance swimmers. *Drug Metab Rev.* 1982;13(3):499-516.
- Bingham, E.; Cofrancesco, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. 4:1335
- DHHS/NTP; Toxicology and Carcinogenesis Studies of Melamine (CAS No. 108-78-1) in F344/N Rats and B6C3F1 Mice (Feed Studies) Technical Rpt Series 245 (1983) NIH Pub 83-2501.
http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr245.pdf read at 17 Sept 08
- Dobson RLM et al (2008). Identification and Characterization of Toxicity of Contaminants in Pet Food Leading to an Outbreak of Renal Toxicity in Cats and Dogs . *Toxicological Sciences Advanced publication*
- IARC. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Geneva: World Health Organization, International Agency for Research on Cancer, V73
<http://monographs.iarc.fr/ENG/Monographs/vol73/mono73-17.pdf> read at 17 Sept 08
- IUCLID (2000) Datasheet on Melamine <http://ecb.jrc.ec.europa.eu/esis/> read at 24 Sept 08
- Lipschitz WL, Stokey E (1945) The Mode of Action of Three New Diuretics: Melamine, Adenine and Formoguanamine *Journal of Pharmacology and Experimental Therapeutics*, Vol. 83, pages 235-249, 22 references, 1945
- Mackenzie, HI, and van Rensburg, I. 1968. Ammelide and ammeline as nonprotein nitrogen supplements for sheep. *J. S. Afr. Veter. Med. Assn.* 39: 41-45.
- Melnick RL et al (1984) Urolithiasis and bladder carcinogenicity of melamine in rodents; *Toxicol Appl Pharmacol* 72 (2): 292-303



OECD (1998) Screening Information Data Set for Melamine, CAS No. #108-78-1 read at <http://www.chem.unep.ch/irptc/sids/OECDSEIDS/108781.pdf>, 17 Sept 08

OECD (1999) Screening Information Data Set for Isocyanuric acid CAS No 108-80-5 read at <http://www.chem.unep.ch/irptc/sids/OECDSEIDS/108805.pdf> 17 Sept 08

Ogasawara H, Imaida K, Ishiwata H, et al (1995). Urinary bladder carcinogenesis induced by melamine in F344 male rats: correlation between carcinogenicity and urolith formation. *Carcinogenesis*; 16(11):2773-7

Pesticide residues in food - 2006. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and WHO the Core Assessment Group. FAO Plant Production and Protection Paper, 187, 2006.

WHO 2004: Evaluation of certain food additives and contaminants (Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 922, 2004.