



## **Expert meeting to review toxicological aspects of melamine and cyanuric acid**

**In collaboration with FAO  
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#### **1 Chemistry of melamine and its analogues**

Melamine is produced in large amounts (1.2 million tonnes in 2007) primarily for use in the synthesis of melamine formaldehyde resins for the manufacture of laminates, plastics, coatings, commercial filters, glues or adhesives, and dishware and kitchenware. Analogues (cyanuric acid, ammeline and ammelide) can be produced as impurities during the manufacturing process for melamine. They may also be produced by the bacterial metabolism of melamine if the melamine is not completely metabolized to ammonia and carbon dioxide.

Melamine can form self-associating, high molecular weight complexes through intramolecular networks of hydrogen bonds and  $\pi$ - $\pi$  aromatic ring stacking interactions with cyanuric acid and other analogues as well as uric acid and other cyclic imide-containing biomolecules. The melamine added to adulterated milk for at least some of the infant formula produced in China that caused renal illnesses during the 2008 incident appeared to be relatively pure. Chinese infant formula reportedly contained levels of cyanuric acid, ammeline and ammelide that were only about 0.1% of the melamine levels and much lower than levels present in contaminated wheat gluten and rice protein concentrate ingredients that were used in the production of pet foods during the 2007 melamine contamination incident in the United States, Canada and South Africa.

### *Recommendations*

- Determine the melamine, uric acid and co-contaminant analogue (cyanuric acid, ammeline and ammelide) residue profiles in renal stones from impacted Chinese infants, and determine the melamine and analogue content of the infant formula samples they consumed, as available.
- Characterize the solubility properties, including thresholds for precipitation, of complexes formed between melamine and the oxytriazine species detected in contaminated foods (cyanuric acid, ammelide and ammeline) and uric acid in vivo and in vitro.

## **2 Methods of analysis of melamine and its analogues in food and feed**

Even though there are various methods currently in use for the screening, confirmation and quantification of melamine and its analogues, they have not been evaluated in an interlaboratory collaborative exercise. Thus, laboratories must thoroughly verify the performance of their method of choice for its intended purpose under their own laboratory conditions.

Liquid chromatography with tandem mass spectrometry and gas chromatography with tandem mass spectrometry are the techniques of choice for confirmatory analysis of melamine and its analogues because of their very high selectivity and high sensitivity.

### *Recommendations*

- Develop and thoroughly validate rapid, simple and low-cost screening methods that can detect melamine plus its analogues in food and feed. Ideally, the new screening tools can be used outside of laboratory settings by various personnel.
- Highly encourage all laboratories involved in melamine analysis to participate in organized proficiency testing programmes for the analysis of melamine and its analogues in food and feed. FAO/WHO should investigate ways of encouraging and facilitating such programmes.
- Develop more specific, rapid and low-cost methods for protein analysis that do not include non-protein nitrogen in order to monitor for, and ultimately prevent, adulteration with sources of non-protein nitrogen.

## **3 Occurrence data**

For this report, the sources of melamine have been divided into “baseline” levels, which refer to levels in food that do not result from adulteration or misuse, and “adulteration” levels, which refer to levels in food that result from the intentional addition of melamine to food or unapproved use or misuse of melamine or substances that can degrade to form melamine.

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. Although data for baseline levels of melamine originating from certain sources, such as migration from tableware, were available, melamine occurrence data from other sources, such as the use of the pesticide cyromazine or of fertilizers, were either limited or unavailable. Data on the presence of melamine and its analogues in other potential sources, such as water from the industrial uses/manufacturing of melamine, were unavailable.

Following the incident related to adulteration of infant formula in China, worldwide government laboratories analysed samples of products containing milk and/or milk-derived ingredients sourced outside of China. Very limited data from the food industry were provided for this review. Some of the reported levels of melamine in foods did not specify a limit of detection or limit of quantification for the analytical method, but rather provided a limit of reporting based on a screening level for melamine in food determined for risk management purposes (1.0 and 2.5 mg/kg). Therefore, from the data available, the distinction between baseline levels of melamine and adulteration levels can be problematic.

Baseline levels of melamine and cyanuric acid may be present in animal feed as a result of the correct use of pesticides, veterinary drugs and feed additives, where such use is approved. Melamine concentrations in animal feed above baseline levels would be the result of misuse or adulteration. Data showing the presence of melamine in animal tissue (including fish), milk and eggs demonstrate that carry-over from feed to tissues, milk and eggs is occurring. However, at this time, inconsistencies in a limited number of data sets do not allow a proper estimation of a feed-to-tissue (milk, eggs) transfer ratio.

#### *Recommendations for data collection and reporting*

- Ensure that FAO/WHO and their member states have effective systems to collect, collate and report data for FAO/WHO data calls and the International Food Safety Authorities Network (INFOSAN).
- FAO/WHO should consider setting up a structured collaboration with relevant food and other industries involved in the food-chain that can be used to generate and/or request data quickly.
- Governments should be encouraged to publish and disseminate data on all testing results (positives and non-detects) in a timely manner, where possible.
- Results should be reported for melamine and its three analogues (cyanuric acid, ammeline, ammelide) in food, feed and feed ingredients, rather than for melamine only.

#### *Recommendations for future work*

Further investigate:

- levels of melamine resulting from the use of cyromazine and other triazine compounds as a pesticide or veterinary drug;
- the extent of use of melamine and analogues as an approved use of additive for animal feed and in fertilizer for different countries to determine expected baseline levels;
- the carry-over ratio of melamine and analogues present in animal feed containing these compounds into food of animal origin (including fish);
- the background levels of melamine present in food products as a result of migration from food contact materials, cross-contamination during processing, processing facilities, packaging material and/or residues of sanitizers;
- the background levels of melamine and cyanuric acid in drinking-water;
- the presence of high levels of melamine in suspect foods other than milk-based products and products containing milk-derived ingredients, such as ammonium bicarbonate and non-dairy creamers.

## 4 Exposure assessment

Currently available melamine occurrence values cover a wide range of concentrations, which increases the difficulty in selecting the appropriate values for use in a dietary exposure assessment.

The Expert Meeting considered that it was not appropriate to sum the dietary exposure assessments from different sources, since the individual exposure assessments were generally very conservative. In addition, a consumer is very unlikely to be exposed simultaneously to the different sources of exposure.

The estimated dietary exposure resulting from baseline levels of melamine in food ranged from 0.0019 to 13 µg/kg body weight per day, depending on the source of the melamine. The migration of melamine from plastics resulted in the highest estimated dietary exposure; however, this estimate is conservative, since it is based on concentrations arising from harsh experimental conditions rather than on measured levels in food as consumed. For cyanuric acid, an exposure estimate of 70 µg/kg body weight per day has been referenced, based only on very conservative assumptions relating to the use of sodium dichloroisocyanurate to disinfect drinking-water.

The dietary exposure resulting from adulteration of Sanlu infant formula based on the median melamine concentrations were 8.6–23.4 mg/kg body weight per day. Estimated dietary exposure to melamine for adults from foods (other than infant formula) containing adulterated milk powder were 0.16–0.7 mg/kg body weight per day, assuming it was present in all food groups with the highest level reported for a food in that group and based on European food consumption data.

### *Recommendations*

- Once additional occurrence data become available, the above dietary exposure assessment should be refined.
- Governments should be encouraged to publish and disseminate dietary exposure estimates in a timely manner, where possible.

## 5 Toxicological and epidemiological data

Melamine and cyanuric acid are rapidly absorbed and excreted unmetabolized in the urine of monogastric animals. The target for melamine or cyanuric acid toxicity is the urinary system in humans and animals. A consistent effect observed with melamine in experimental animals is bladder stones, with some studies observing microcrystalluria. Some bladder stone formation has also been reported following exposure to cyanuric acid. Carcinogenic effects observed with melamine are considered to be secondary to irritation caused by stones. Melamine co-exposure with cyanuric acid can induce acute melamine–cyanurate crystal nephropathy, leading to renal failure at much lower doses than with either compound given individually. There are very few data on melamine analogues other than cyanuric acid.

Data from the 2008 Chinese incident illustrate that infant formula contaminated mainly with melamine can result in stone formation if sufficient concentrations are present. Limited data indicate that stones are composed of uric acid and melamine at a molar ratio ranging from 1.2:1 to 2.1:1, without evidence of the presence of cyanuric acid or other melamine analogues.

Although limited data are available on the concentrations of melamine and related compounds in the adulterated infant formula consumed by affected infants, they were deemed insufficient for the development of meaningful or realistic exposure estimations.

Most children with stones did not have clinical signs of illness. However, in severe cases of renal failure and/or blockage, clinical signs did occur. Treatment has been symptomatic and supportive and depends on the severity of clinical signs. Therapeutic interventions have included oral fluid (water) administration, intravenous fluid administration, alkalization of urine (to reach urine pH of 6.5–7.0) with bicarbonate or citrate, haemodialysis, peritoneal dialysis and surgical removal of stones. The prognosis was considered good for infants who received treatment.

These human data are different from what has been described for the outbreaks in pets in 2004 and 2007. From all data reported to date, infants were exposed primarily to melamine alone or to very low levels of cyanuric acid when melamine was present at very high concentrations, whereas pets were exposed to melamine and cyanuric acid and possibly to ammeline and ammelide. Affected infants developed stones in the urinary tract, whereas pets developed crystals in renal tubules and subsequent obstructive nephropathy. Pets developed acute renal failure within 2 days of exposure in severe exposures, whereas most infants with stones did not have overt clinical symptoms.

#### *Recommendations for information dissemination*

- In order to facilitate obtaining needed data, the Expert Meeting recommends that research groups communicate and coordinate efforts worldwide. WHO and FAO may serve as good platforms for disseminating this information.

#### *Recommendations for further research*

The following recommendations should be considered in the context of experimental dosing that involves doses ranging from baseline to adulteration levels:

- Determine the threshold dose and time course for crystal development in the kidney and urine for melamine alone and in combination with cyanuric acid and other triazines in different molar ratios, and study the influence of pH on the solubility of melamine-induced stones for treatment purposes. The group is aware of studies under way or planned by the United States Food and Drug Administration in swine and rats to determine a threshold for combined exposure to cyanuric acid and melamine. Studies in cows are also planned at the University of California–Davis, in the Republic of Korea and in South Africa.
- Perform studies to better understand melamine toxicokinetics with models that reflect uric acid levels in humans, especially neonates. Comparative toxicokinetics in humans and other species should also be studied.
- Conduct research into the contribution of various risk factors (e.g. reduced kidney function, age, influence of medications such as diuretics) to toxicity.
- As formalin dissolves melamine crystals, other tissue preservation techniques should be used in order to detect melamine crystals in tissue preparations. Research is needed on the solubility of other triazine crystals in formalin.
- Determine whether biomarkers and diagnostic techniques can be identified for predicting renal damage following exposure to melamine-type compounds. Develop urine tests for whole population studies that could be used as biomarkers to indicate potential problems

prior to stone formation (e.g. the detection of crystals of melamine–cyanuric acid or melamine–uric acid in urine). It should be noted, however, that owing to their short half-life, small molecule size and rapid excretion within hours of consumption of food or water containing melamine or its analogues, very sensitive methods will be required to measure the low levels of crystals that may be present in urine. There will also be a need to sample urine from a large population in order to increase the chances of obtaining a representative view of such a biomarker.

- Conduct studies to determine if in utero exposure occurs and the extent of exposure from human milk. Conduct studies to investigate potential reproductive and developmental effects.
- Conduct studies to understand the mechanism of toxicity and subtle renal alterations induced by subchronic low doses of melamine or exposure to short intermittent high doses. Design studies to model the 2008 incident to seek biomarkers of long-term effects (see above) following early-life melamine exposure in humans that could inform the epidemiological investigation (see below).
- Perform long-term follow-up of infants who had stones in terms of renal function and cancer incidence.

## **6 Derivation of the TDI**

Epidemiological studies conducted on affected infants show that infants exposed for the longest time and to the highest concentrations of melamine were at highest risk of developing kidney stones. However, the data available do not allow a detailed dose–response assessment. As a result, it is necessary to rely on suitable toxicological studies in laboratory animals for risk assessment purposes.

Two 13-week studies in young rats administered melamine in the diet were selected as the most relevant studies for evaluation. A benchmark dose approach (dose-response modelling) was applied to these data using three model types. As there have been sporadic findings of bladder stones in experimental rats, including a 4.5% incidence in the control male rats from the subchronic studies, it was determined that a lower limit on the benchmark dose for a 10% response rate (the BMDL<sub>10</sub>) would be an appropriate toxicological end-point. The BMDL<sub>10</sub> was calculated to be 35 mg/kg body weight per day. It is noted that this value is conservative, as no bladder stones were observed in weanling rats exposed to melamine in the diet at a dose of about 168 mg/kg body weight per day for 4 weeks.

This BMDL<sub>10</sub> can be used to estimate the tolerable daily intake (TDI). Applying a safety factor of 200 to this value to account for extrapolation from rats to humans, variation within humans and uncertainties associated with the data results in a TDI of 0.2 mg/kg body weight per day (rounded to one significant number).

This TDI is applicable to exposure to melamine alone. Although data were inadequate to develop TDIs for compounds that are structurally related to melamine, such as ammeline and ammelide, a TDI of 1.5 mg/kg body weight for cyanuric acid has been previously derived by WHO, suggesting that these analogues would be no more toxic than melamine. Available data indicate that simultaneous exposure to melamine and cyanuric acid is more toxic than exposures to each compound individually, although data are not adequate to allow the calculation of a health-based guidance value for this co-exposure. This should be re-evaluated by WHO once more dose–response data on the combined exposures become available.

## **7 Risk characterization**

The dietary exposure based on the consumption of melamine-adulterated infant formula in China at the median levels of melamine reported in the most contaminated brand was estimated to range from 8.6 to 23.4 mg/kg body weight per day, based on data provided by the Chinese Center for Disease Control and Prevention. This is about 40–120 times the TDI of 0.2 mg/kg body weight, explaining the dramatic health outcome in Chinese infants. Conservative estimates of potential exposure of adults to melamine from foods containing adulterated milk products were 0.8–3.5 times the TDI. Estimates of exposure to baseline levels of melamine from all sources (up to 13 µg/kg body weight per day) were well below the TDI.

In developing the TDI, the Expert Meeting specifically considered the sensitivity of infants. The TDI is derived from toxicological studies in weanling rats, and an additional uncertainty factor is applied to take into account some uncertainties in the database. Hence, the TDI is applicable to the whole population, including infants.

Development of a TDI does not mean that adulteration of food to a level consistent with the TDI is acceptable. This applies to the TDI derived above for melamine as well as the TDI developed previously for cyanuric acid.

TDIs are chronic values and are intended to protect over the lifetime of an individual. Occasional modest exceedance of the TDI is not likely to be of health concern. Prolonged exceedances may be of health concern. The amount and duration of exceedance likely to be without effect are compound specific and depend on the circumstances of exposure.

## **8 Risk management**

Many countries have introduced limits for melamine in infant formula and other foods. Limits for melamine in powdered infant formula (1 mg/kg) and in other foods (2.5 mg/kg) would provide a sufficient margin of safety for dietary exposure relative to the TDI.

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