Toxicological and Health Aspects of Melamine and Cyanuric Acid

**Report of a WHO Expert Meeting
In collaboration with FAO
Supported by Health Canada**

Health Canada, Ottawa, Canada
1–4 December 2008

CONTENTS

EXECUTIVE SUMMARY

According to a report from the Chinese Ministry of Health, 294 000 infants had been affected by melamine-contaminated infant formula by the end of November 2008. More than 50 000 infants have been hospitalized, and six deaths have been confirmed. Because of the large potential health impact, the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) convened an Expert Meeting.

Melamine produces crystals in urine when its concentration exceeds a threshold. Exposure below this threshold will generally not result in adverse health effects. Many of the affected infants in the Chinese incident had stones, or calculi, in the kidney, ureter or bladder. These calculi were composed of uric acid (a normal waste product in human urine) and melamine.

Melamine is an industrially synthesized chemical used for a wide variety of applications, such as laminates, coatings and plastics. Commercially produced melamine may contain structural analogues, such as cyanuric acid, ammelide and ammeline.

Humans are exposed to melamine and its analogues from a number of different sources, including food and environmental sources. Sources range from breakdown of the pesticide cyromazine, which is approved for use in many countries, to migration from approved food packaging material to the adulteration of specific foods. A specific source of exposure for which very few data were available is carry-over from the (mostly non-approved) presence of melamine in animal feed or feed ingredients. Data have shown carry-over from feed to products of animal origin (e.g. milk, eggs, meat), including fish.

Methods are available for the screening and quantification of melamine in food and feed. Selective methods are able to detect very low concentrations of melamine and its analogues in such products.

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 the unapproved use or misuse of melamine or substances that can degrade to form melamine.

Adulteration occurs, in part, because commonly used methods for protein analysis cannot distinguish between nitrogen from protein sources and nitrogen from non-protein sources. This results in incorrectly high protein measurements for products containing non-protein nitrogen sources like melamine and provides an economic incentive for their (illegal) addition. New, simple, specific, rapid and cost-effective methods for protein quantification should be developed to discourage adulteration.

The baseline exposure has been estimated from (limited) data on concentrations in different food groups and food consumption data based on the WHO consumption cluster diets and national data. However, industry data on baseline occurrence have generally not been published and were not made available to FAO and WHO, although such data exist for a number of industrial food products. The very limited availability of data seriously hampered the ability of the Expert Meeting to

estimate exposure. The food and feed industries should be encouraged to share data, and FAO and WHO should set up better systems for confidential data sharing.

Because of insufficient human data, it was necessary to rely on toxicological studies in laboratory animals to characterize the human health risk related to melamine in food.

Based on dose–response assessment of subchronic rat studies, modelling of the incidence of bladder stones and application of a safety factor of 200 to account for extrapolation from rats to humans, variation within humans and uncertainties associated with the data, a tolerable daily intake (TDI) of 0.2 mg/kg body weight for melamine was established. The TDI is applicable to the whole population, including infants.

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 previously been 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. Data are not adequate to allow the calculation of a health-based guidance value for this co-exposure.

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.

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.

The Expert Meeting provided a range of recommendations for further information and new studies to better understand the risk to human health posed by melamine and its analogues.

1. BACKGROUND

As of November 2008, there had been six deaths and 294 000 affected infants, more than 50 000 of them hospitalized, with urinary problems, possible renal tube blockages and possible kidney stones related to the consumption of melamine-contaminated infant formula and related dairy products. Several previous melamine contamination incidents had been reported in pet food ingredients or products, one of which led to the recall of 1154 pet food product types and is believed to have caused the deaths of more than 1000 dogs and cats as a result of kidney failure. Melamine has a high nitrogen content (66.6% by weight), which makes it attractive for economic adulteration as a fraudulent substitute for protein, especially when indirect protein assays based on total nitrogen are used.

In light of concern expressed by Member States, an Expert Meeting was convened by the World Health Organization (WHO) in collaboration with the Food and Agriculture Organization of the United Nations (FAO) and with the support of Health Canada. The Expert Meeting was held at Health Canada in Ottawa, Canada, on 1–4 December 2008. A list of participants and the agenda as adopted are provided in Annexes 1 and 2, respectively. Dr Junshi Chen of the Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, served as Chairperson, and Mr Mark Feeley, Bureau of Chemical Safety, Health Canada, served as Rapporteur. All participants signed confidentiality agreements and declarations of interest; no potential or real conflicts of interest were identified.

The Expert Meeting was opened by Dr Janet Beauvais, Director-General of the Food Directorate of Health Canada. She welcomed the participants, expressed her pleasure that Health Canada was able to provide support for the meeting and predicted that the meeting would contribute to current understanding of the chemistry, analysis, occurrence, toxicity and exposure associated with melamine and its structural analogues. Dr Jørgen Schlundt, Director of WHO's Department of Food Safety, Zoonoses, and Foodborne Diseases, thanked the Government of Canada for funding the meeting and providing logistical support, explained the urgent need for the meeting and described how it had been arranged so quickly. He also thanked the participants for generously contributing their time and expertise to WHO.

2. OBJECTIVES OF THE EXPERT MEETING

The objectives of the Expert Meeting were:

1. to review current knowledge on:

   - the chemistry of melamine alone and in combination with its analogues (e.g. cyanuric acid);
   - analytical methods relative to detection of these chemical substances in various foods;
   - the occurrence of melamine in food and feed as a result of normal production and processing (e.g. from migration of food contact material, pesticide or fertilizer use), as opposed to adulteration;
   - the toxicity of melamine alone and in combination with its analogues (e.g. cyanuric acid);
   - estimated dietary exposure from various sources;
   - human health risk assessment, including species sensitivity and sensitive subpopulations;

2. to identify knowledge gaps to guide research efforts.

The Expert Meeting reviewed background documents prepared for the meeting by invited experts as well as additional published and unpublished data provided by meeting participants. These background documents, revised according to discussions at the meeting, can be accessed on the WHO web site (http://www.who.int/foodsafety/en/). Annex 3 contains a list of contributors to those background documents. References cited in the present report are listed in section 10. A list of abbreviations may be found in Annex 4.

3. CHEMISTRY OF MELAMINE AND ITS ANALOGUES

The structures of melamine and related triazine compounds are illustrated in Figure 1.

Melamine   Cyanuric acid   Ammelide   Ammeline

Trichloromelamine   Cyromazine
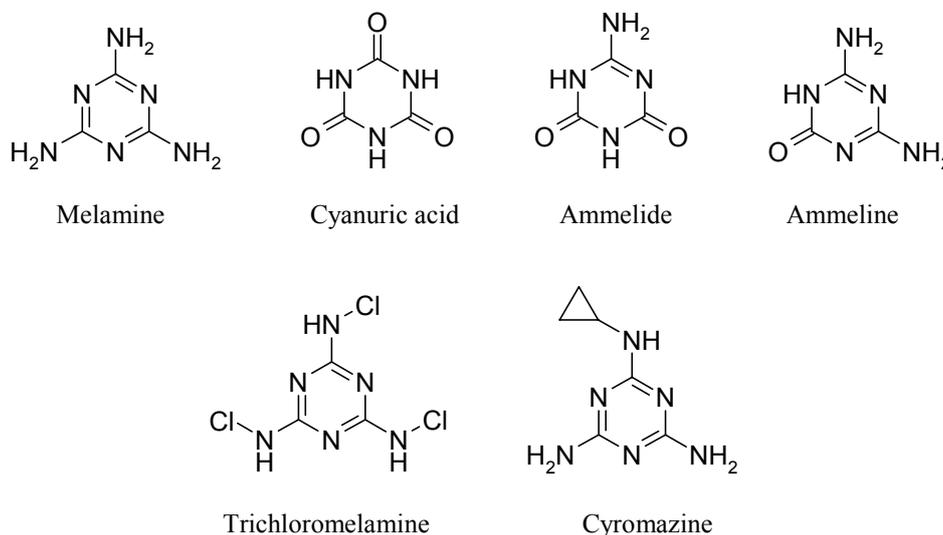
Figure 1. Structures of melamine and related triazine compounds

3.1   Chemistry, production and uses of melamine and related compounds

3.1.1 *Melamine*

World production of melamine (Chemical Abstracts Service [CAS] No. 108-78-1) in 2007 was approximately 1.2 million tonnes, with the predominant producers being located in China and western Europe (Bizzari & Yokose, 2008). 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, and some proprietary processes give product purities as high as 99% (Maxwell, 2007; Bizzari & Yokose, 2008).

Melamine is a nitrogen-rich heterocyclic triazine used primarily in the synthesis of melamine–formaldehyde resins for the manufacture of laminates, plastics, coatings, commercial filters, glues or adhesives, and moulding compounds (dishware and kitchenware) (Bizzari & Yokose, 2008). Melamine is also reportedly used as a colorant (paint coating) and as a fertilizer.

There are no approved uses for the direct addition of melamine to food (e.g. in the United States of America [USA] and Europe). In the USA, melamine is an indirect food additive used in the synthesis of melamine-formaldehyde resins (e.g., 21 CFR 175.300, 175.320 and 177.1010) and as a component of adhesives (21 CFR 175.105, United States National Archives and Records Administration's Electronic Code of Federal Regulations, http://www.gpoaccess.gov/ecfr, as of 18 June 2007). In Europe, melamine is approved for use as a monomer and as an additive in plastics

(Commission Directive EC No. 2002/72 related to materials and articles intended to come into contact with foodstuffs, 6 August 2002).

Adulteration occurs, in part because commonly used methods for protein analysis cannot distinguish between nitrogen from protein sources and nitrogen from non-protein sources. This results in incorrectly high protein measurements for products containing non-protein nitrogen sources like melamine and provides an economic incentive for their (illegal) addition.

The purity of melamine products is highly dependent upon the manufacturing process and the level of purification. Low-purity melamine-containing solids can be recovered from the mother liquor wastewater stream following the crystallization stage in the commercial production of melamine. Analysis of one such wastewater stream having a total solids percentage of 1.80% yielded melamine at 1.27% of the total solids and oxytriazines (including ammeline, ammelide and cyanuric acid) at 0.42% of the total solids (Ono et al., 1998). It is possible that solids from the mother liquor wastewater stream are representative of the "melamine scrap" discussed in many news articles related to melamine adulteration in pet food in 2007.

Very recent Chinese data (Chinese Center for Disease Control and Prevention, unpublished data, 2008) on the analysis of 15 raw materials that were used for the adulteration of milk in Gansu province, China, which was then used to make infant formula, indicated that the median levels of melamine and related compounds in the raw materials were as follows: melamine (188 000 mg/kg), cyanuric acid (3.2 mg/kg), ammeline (14.9 mg/kg) and ammelide (293 mg/kg). The melamine used to adulterate milk that was then used for at least some of the infant formula in the current 2008 incident appears to be of higher purity, with much lower levels of cyanuric acid, ammeline and ammelide, than that used in the wheat gluten and rice protein concentrate ingredients that were used in the production of pet foods during the 2007 melamine contamination incident in the USA, Canada and South Africa.

In a study of the effectiveness of triazines as fertilizers, it was demonstrated that melamine can be metabolized by at least two strains of bacteria occurring in soil (*Pseudomonas* strain A and *Klebsiella terragena*) through successive deamination reactions to form ammeline, ammelide and cyanuric acid, with further breakdown to biuret, urea and, ultimately, ammonia and carbon dioxide (Jutzi, Cook & Hutter, 1982; Shelton et al., 1997).

3.1.2 Related triazine compounds

Trichloromelamine (CAS No. 7673-09-8), which decomposes to melamine, is regulated in the USA and other countries for use in sanitizing solutions used on food processing equipment, utensils and other food contact articles, with the exception of milk containers or equipment. In addition, the United States Environmental Protection Agency (USEPA) allows the use of trichloromelamine as a sanitizer and disinfectant on hard surfaces and as a component of a wash solution for fruits and vegetables.

Melamine is a metabolite of the pesticide *cyromazine* (CAS No. 66215-27-8) (FAO, 2007b). However, it is known that cyromazine on the surface of fruits and vegetables is converted to melamine over time (Lim et al., 1990; USEPA, 1999).

Cyanuric acid (CAS No. 108-80-5) is an oxytriazine melamine analogue that may be produced as a by-product in melamine synthesis. In the USA, it is accepted as a component (up to 30%) of feed-grade biuret, a ruminant feed additive (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=573.22 0). It is also found in drinking-water and in swimming pool water as the dissociation product of dichloroisocyanurates used for water disinfection (WHO, 2004, 2007). When used for disinfection purposes in water, sodium dichloroisocyanurate is rapidly

hydrolysed to form free available chlorine and cyanurate (Brady, Sancier & Sirine, 1963; Matte et al., 1990). Cyanuric acid derivatives are regulated in the USA as components of sanitizing solutions for use on food processing equipment, utensils and other food contact articles (USFDA, 2003a).

Ammelide (CAS No. 645-93-2) and *ammeline* (CAS No. 645-92-1) are, respectively, monoamino- and diaminooxytriazine analogues produced as by-products of melamine synthesis or by the microbial degradation of melamine. Ammeline is used in lubricating greases (USFDA, 2007). No information on the uses of ammelide was available.

3.2   Structures and properties of melamine complexes

Melamine and related triazines are able to form self-assembling, high molecular weight complexes (see Figure 2 for a simplified melamine–cyanurate complex) via organized intramolecular networks of hydrogen bonds and π-π aromatic ring stacking (Seto & Whitesides, 1990; Whitesides, Mathias & Seto, 1991). Biomolecules possessing similar cyclic imide structures with the ability to form hydrogen bond networks are common. Uracil, riboflavin, barbituric acid and uric acid each possess imide groups known to interact with melamine in self-associating complexes (see Figure 3 for a simplified melamine–urate complex). Intermolecular complexes with ammeline and ammelide have not been studied in similar detail.
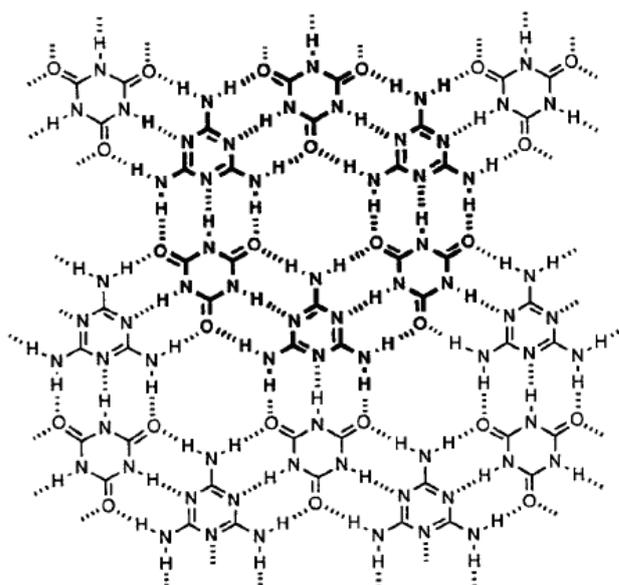
Figure 2. Melamine–cyanuric acid lattice (cyclic hexamer shown in bold) (Sherrington & Taskinen, 2001) *Reproduced by permission of The Royal Society of Chemistry*
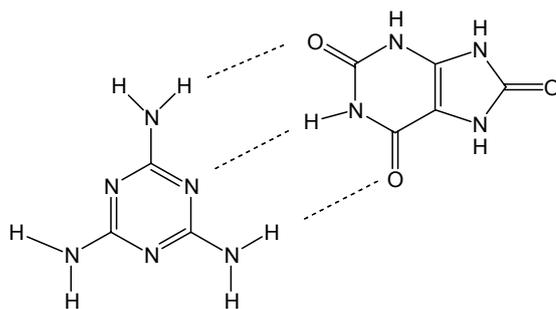
Figure 3. Melamine–urate complex (simplified and hypothetical) (adapted from Reimschuessel, 2008)

Triazine hydrogen bond networks may be subject to disruption by acid–base equilibria or high temperature. The effect of pH on the solubility of the melamine–cyanurate hydrogen-bonded complex was recently determined (Figure 4) (Tolleson, 2008). As predicted from the acid dissociation constants for the melaminium cation ($C_3H_7N_6^+$) and cyanuric acid, minimum solubility for the complex was observed under conditions favouring the co-existence of melamine free base and non-ionized cyanuric acid (pH 5.0). The solubility of the complex increased markedly as the pH decreased to pH 3.5 and the concentration of melaminium cation increased. By contrast, the solubility of melamine–cyanurate increased marginally as the pH increased to pH 7.5. Interestingly, spherulite crystals of melamine–cyanurate that formed within the urinary tracts of fish and pigs were shown to dissolve in formalin over a period of hours but in acid in a period of minutes (Reimschuessel et al., 2008).
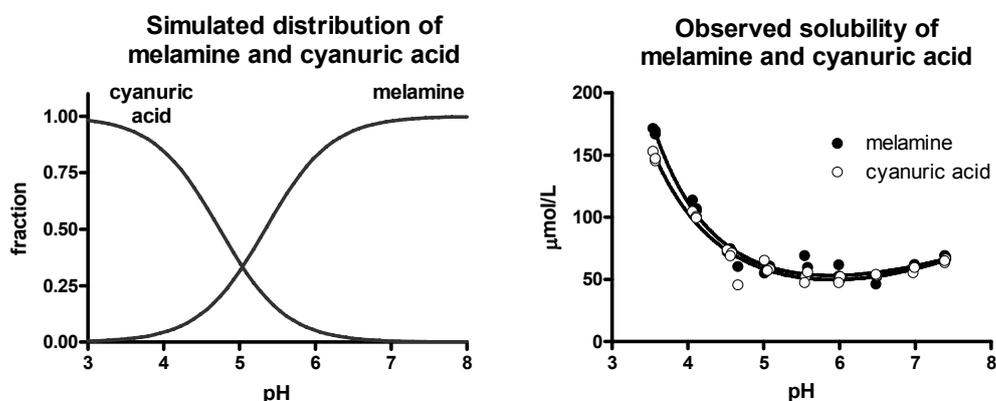
Figure 4. Influence of pH on solubility of melamine–cyanurate

Because melamine exhibits intrinsic fluorescent properties (excitation maximum 255 nm, emission maximum 390 nm), melamine titrations can be monitored fluorimetrically. Using this approach, the melamine–cyanurate complex exhibited a dissociation constant (K_d) of 31 ± 7 µmol/l in water (W. Tolleson, personal communication, 2008). For comparison, the transition metal ions copper(II), iron(II) and zinc(II) exhibited much weaker binding to melamine (K_d 1.8, 1.0 and 30 mmol/l, respectively). Fluorescence titrations were also used to evaluate the effect of pH on the stability of melamine–urate complexes (Figure 5). At neutral pH, the affinity of melamine for uric acid was found to be 29-fold weaker than that for cyanuric acid. Melamine–urate exhibited up to 6.3-fold tighter binding under acidic conditions in comparison with its affinity at pH 7.0 (K_d 140, 240, 490 and 900 µmol/l

at pH 4.0, 5.0, 6.0 and 7.0, respectively). Chickens, which normally excrete larger amounts of uric acid than mammals, were exposed to melamine in preliminary United States Food and Drug Administration (USFDA) studies. These studies showed that spherulites, presumably composed of melamine–urate crystals, dissolved rapidly in formalin fixative (R. Reimschuessel, personal communication, 2008).
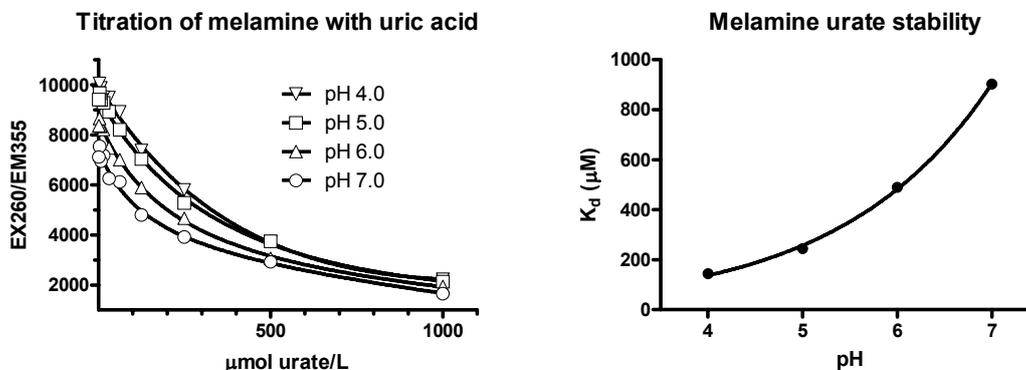
Figure 5. Influence of pH on melamine–urate dissociation

The chemical composition of renal calculi found in victims of melamine intoxication is an issue of critical importance. Some anecdotal descriptions of "sludge-like" or "sand-like" urinary precipitates observed in infants exposed to melamine-contaminated milk products are considered reminiscent of certain uric acid–containing uroliths that could possibly contain melamine. Very recent Chinese data on the composition of renal calculi (stones) from 15 infants in China who had consumed contaminated infant formula (Chinese Center for Disease Control and Prevention, unpublished data, 2008) confirmed that these stones were composed of uric acid and melamine (in a 1.2:1 to 2.1:1 molar ratio), and no cyanuric acid was detected.

4. METHODS FOR THE ANALYSIS OF MELAMINE AND ITS ANALOGUES IN FOOD AND FEED

4.1 Review of methods of analysis

Existing methods for the analysis of melamine and its analogues (ammeline, ammelide, cyanuric acid, melamine–cyanurate) in foods and animal feeds were reviewed. These methods include rapid screening and selective quantitative methods. Some are multiresidue methods and can be used to analyse for multiple compounds during one process.

4.1.1 Sample preparation

As melamine may not be uniformly present in the sample, it is advisable that the samples be properly homogenized prior to subsampling and extraction.

4.1.2 Sample extraction

Initially, liquid extraction with an acidic aqueous solvent mixture is used to extract samples. For more sensitive detection methods, liquid extraction is often followed by further cleanup with mixed-mode solid-phase extraction to avoid interferences from co-extracted matrix components. Proper selection of the solid-phase sorbent is required for multiresidue methods. Cation exchange/reversed phases are used in the analysis of melamine (Andersen et al., 2008), whereas cyanuric acid is isolated using mixed-mode anion exchange sorbents (Smoker & Krynitsky, 2008). Other solid-phase sorbents used to further process sample extracts include graphitized carbon phases (Patakioutas et al., 2007; Karbiwnyk et al., 2008) and C_{18} (Chou, Hwang & Lee, 2003).

Because melamine may also form relatively less soluble complexes with cyanuric acid and uric acid under certain conditions, the pH of the extraction solvent may need to be modified to adequately dissociate and extract the complexes. Acidic extraction solvents incorporating hydrochloric acid, formic acid or acetic acid have been used for methods analysing melamine and cyanuric acid (Turnipseed et al., 2008). However, it has been noted that a solution of diethylamine/acetonitrile/water also effectively dissolves melamine–cyanurate (Filigenzi et al., 2008). On the other hand, melamine–urate is less stable at higher pH (see Figure 5 above).

In addition, there is a possibility of hydrolysis of melamine and its analogues to cyanuric acid. Therefore, care should also be exercised if using extremely basic conditions during sample extraction and analysis.

4.1.3 Detection and quantification

Characteristics of the techniques currently in use to detect and quantify melamine and its analogues in foods and feed are summarized in Table 1. The relative selectivity, sensitivity and cost of the various techniques are indicated as well. Brief discussions of the various techniques follow.

Table 1. Analytical techniques used for analysis of melamine and analogues in food and feed

Analytical technique	Selectivity	Sensitivity	Cost	Purpose	Remarks
ELISA	Low	Low	Low	Screening	Currently for detecting melamine only
HPLC-UV/DAD	Low	Low	Medium	Screening and confirmation	Preferred for screening of melamine and analogues; however, validation is required for confirmation
GC-MS	Medium	Medium	High	Screening and confirmation	Confirmatory method for melamine and analogues
GC-MS/MS	High	High	Very high	Screening and confirmation at trace levels	Confirmatory method for melamine and analogues
LC-MS/MS	High	High	Very high	Screening and confirmation at trace levels	Confirmatory method for melamine and analogues

DAD, diode array detection; ELISA, enzyme-linked immunosorbent assay; GC, gas chromatography; HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; UV, ultraviolet.

Enzyme-linked immunosorbent assay (ELISA) methods must be validated for matrices other than those validated by the ELISA kit manufacturers using a confirmatory method. Positive results obtained using an ELISA for the analysis of melamine must be confirmed using a more selective confirmatory method. Reported quantification limits for melamine in currently available ELISAs range from 0.1 to 25 mg/kg and depend upon the matrix being analysed and the sample extraction method used.

High-performance liquid chromatography–ultraviolet/diode array detection (HPLC-UV/DAD) should be used in confirmatory methods only if it has been validated thoroughly for the matrices of interest; otherwise, it can be used only as a screening method. As with ELISA screening methods, positive results obtained using HPLC-UV/DAD as a screening method must be confirmed using a more selective confirmatory method. Reported quantification limits for melamine and analogues for all existing HPLC-UV/DAD methods range from 0.05 to 65 mg/kg and depend upon the matrix being analysed and the sample extraction method used.

Gas chromatography–mass spectrometry (GC-MS) methods can be used as a confirmatory and/or a screening method due to their medium selectivity. Owing to the small, polar nature of melamine and its analogues, trimethylsilyl derivatives are produced for GC analysis in the electron impact ionization mode. At least three ions should be monitored to prove the identity of target compounds. Reported quantification limits for existing methods range from 0.05 to 10 mg/kg and depend upon the matrix being analysed and the extraction method used.

Tandem mass spectrometry (MS/MS)-based methods can also be used as a confirmatory and/or a screening method due to their high selectivity. Most MS/MS methods monitor at least two ion transitions for each analyte to ensure the unambiguous identification of the target analytes. This is preferred, so that ratios of transitions can be calculated and used as another identification criterion for analytes. For HPLC-MS/MS, melamine is analysed in the positive electrospray ionization mode; analogues are analysed in the negative ionization mode. The different polarities required necessitate the use of polarity switching during one HPLC-MS/MS run and,

thus, adequate analyte chromatographic separation or two different HPLC-MS/MS runs. Trimethylsilyl derivatives are produced for GC-MS/MS analysis of melamine and analogues. Analysis is performed in the electron impact ionization mode. Reported quantification limits for melamine and analogues range from 0.002 to 5 mg/kg and depend upon the matrix being analysed and the extraction method used.

4.1.4 Validity of analytical data

To ensure the validity of analytical data, laboratories must verify the performance of their method of choice for its intended purpose under their own laboratory conditions. This is particularly important considering that background contamination has been observed and may be related to each laboratory's equipment and supplies being used.

In addition, confirmatory methods must use internal standards to negate effects on quantification caused by the sample matrix. Matrix effects have been observed to occur for a variety of matrices and can result in interferences with melamine or analogues during detection and quantification. For MS-based methods, it is highly recommended that stable isotope–labelled internal standards be used for melamine and cyanuric acid (plus other analogues, should they become available). For UV-based methods, it is highly recommended that a suitable non-stable isotope–labelled internal standard be used. For example, resorcine was added prior to extraction of rice protein concentrate and animal feeds in Muniz-Valencia et al. (2008). Atrazine has also been used to compensate for variations during injections in the analysis of bean plants (Patakioutas et al., 2007). The use of hydrophilic interaction chromatography can improve the resolution of melamine and analogues and avoid co-elution of analytes and matrix components for methods using HPLC as well.

4.2 Development of an analytical strategy for measurement of melamine and its analogues

There are a number of factors that any organization or group that is planning to begin analysing melamine and its analogues should take into consideration. These considerations deal with the choice of analytical method(s) to use and the steps required to ensure that valid and accurate data are produced.

4.2.1 Selection of analytical method

There are many developed analytical methods to choose from. The selection of the most appropriate method will be driven by the overall goal of the analytical strategy; ultimately, the method chosen must be fit for its intended purpose. More specifically, analytical method selection will be based on the sensitivity and selectivity required, as well as the instrumentation and personnel resources available in the laboratory that will be undertaking the analyses.

Compliance testing or the screening of samples for the identification of adulterated products can be carried out using less sensitive and/or selective methods, such as ELISA or HPLC-UV/DAD-based methods. More sensitive MS-based methods can also be used but are not required, as they are for trace analysis. Care must be taken to ensure that the chosen methods can reliably detect melamine or its analogues at the levels of interest (such as a maximum level) in the appropriate matrices. In addition, there must be an accompanying confirmatory method available

for the confirmation of positive samples. Table 1 in section 4.1.3 indicates the analytical techniques available for use in confirmatory methods.

If there is a high likelihood that samples to be analysed contain violative levels (i.e. levels above regulatory limits) of melamine or analogues, the use of a selective confirmatory method at first, as opposed to a screening method, should be considered. This will require a validated HPLC-UV/DAD, GC-MS, GC-MS/MS or LC-MS/MS method.

Trace analysis for the determination of low baseline levels requires the use of GC-MS/MS- or LC-MS/MS-based methods, because they provide the most sensitivity.

Analysis of samples for analogues of melamine, such as cyanuric acid, ammeline or ammelide, requires the use of a multiresidue method. Currently, only UV- and MS-based methods are multiresidue methods.

Many sample extraction and cleanup methods exist and are publicly available. They cover analysis of a variety of matrices, from liquid and powdered infant formula, dairy products, biscuits, cakes and confectionery to fish and seafood. Method development is still ongoing, as surveillance activities are expanding to include other food items, such as cereal products, food processing ingredients, animal feed and soy-containing products. Thus, it is not prudent to highlight specific methods for matrices at this time, as progress is still being made in refining methods. However, it is worthwhile to state that since most extraction and cleanup methods are generic as opposed to matrix specific, their application to novel matrices may not require much modification.

4.2.2 Validation

Method users must independently evaluate any method chosen as part of an analytical strategy to ensure that it works according to their needs and in their laboratories. This is particularly important considering that validation data are not readily available for many of the methods described in the literature. In addition, contamination has been reported during some analyses and may be related to the laboratories' physical surroundings and equipment and supplies being used. Modifications can often be made to improve existing methods for specific analyses as well.

4.2.3 Quality control during analyses

To ensure that valid and accurate data are produced, all analytical strategies should incorporate typical analytical quality control procedures to minimize and control for confounding or interfering factors during analyses. In particular, blanks and internal standards must be used to monitor for background contamination and matrix effects (as described in section 4.1).

Currently, there are no certified reference materials available for melamine or its analogues. However, two organizations have thus far organized proficiency tests using melamine-contaminated pet food (FAPAS®, http://www.fapas.com/fapas.cfm) and milk powder (Institute for Reference Materials and Measurements, http://irmm.jrc.ec.europa.eu/html/homepage.htm). Samples remaining from these tests may be used as uncertified reference materials. Laboratories can also prepare their own in-house reference materials from items found to contain melamine or analogues during surveillance and monitoring activities or from blank items fortified with melamine or analogues.

4.2.4 Sampling plans

As with the selection of the analytical method, the development of a sampling plan depends upon several factors, including the goal of the testing strategy and the resources available.

At a minimum, the items selected for testing should include those that have been found to contain melamine or analogues at levels above the regulatory limits in other jurisdictions, and an appropriate number of samples for each item should be included. Some national food safety organizations have posted lists of these items on their respective web sites, and compiled lists have also been distributed through the emergency network of the International Food Safety Authorities Network (INFOSAN) (http://www.who.int/foodsafety/fs_management/infosan/en/index.html).

After the infant formula adulteration incident in China, the initial focus of sampling for many testing strategies was on Chinese dairy products and other foods that contained dairy ingredients manufactured in or sourced from China. Subsequent sampling by some countries was expanded to include other products originating from China that may have been exported to various countries (including fruits and vegetables, egg products, non-dairy creamers, ammonium bicarbonate and animal feed).

Testing strategies of some organizations have also been expanded to include products similar to those initially found to contain elevated levels of melamine or analogues. These items include not only those made by different manufacturers from areas of interest, such as China, which may also contain contaminated ingredients, but also similar products manufactured in other areas.

More thorough testing strategies would also include sampling and analysis of locally produced items analogous to those manufactured in or sourced from China and other areas that have been reported to contain relatively high levels of melamine and related compounds. Selection of these locally produced items that are readily available and/or consumed by the local population can be guided by consumption information, if available from market data or food consumption surveys. Convenience sampling, in which items that are readily available to the local population in marketplaces are sampled, can also be used in a sampling plan as a means of selecting locally produced items for analysis.

A more complete picture of dietary exposure to melamine and its analogues will require the selection and analysis of a wider variety of food items that cover a larger proportion of the diet. Such a sampling plan will capture food and feed items that are not necessarily adulterated, but may still contribute to a population's baseline exposure to melamine and its analogues. Further details on the occurrence of melamine and its analogues at baseline and adulterated levels in various food and feed items can be found in section 5 of this report. Sampling and analyses of a wider variety of food or feed items to determine baseline exposure will require use of a more sensitive analytical method.

Regardless of the sampling plan employed in a testing strategy, as much information as possible on the samples selected for analysis should be recorded. This will aid in the tracing back of the source of a food or feed item, should the need arise.

5. OCCURRENCE OF MELAMINE AND ITS ANALOGUES IN FOOD AND FEED

5.1   Definitions of baseline, adulteration and misuse

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, including misuse, which refer to the intentional addition of melamine to food or unapproved use of melamine or substances that can degrade to form melamine.

Baseline is defined as levels of melamine and related analogues in food from accepted uses that do not result from adulteration or misuse. This includes expected levels from the environment, food processing, packaging materials, residues from the legitimate use of triazine pesticides or veterinary drugs, and legitimate use of melamine in fertilizers or of cyanuric acid in feed additives.

Adulteration is the intentional addition of melamine and/or analogues directly to food, food ingredients, animal feed, feed ingredients or pelletizing agents. It may also be present indirectly in foods of animal origin as a result of carry-over from the intentional addition to animal feed.

Misuse is defined as the inappropriate use of cyromazine (a pesticide that metabolizes to melamine) or biuret (a ruminant feed additive containing cyanuric acid) in animal feed or use of animal feed containing these additives in species for which it is not intended.

5.2   Sources and occurrence of melamine and related analogues

Melamine and its analogues may be present in the environment or in the food-chain as a result of their legitimate widespread uses (see section 1.3) or as a result of the degradation of precursor compounds. Legitimate uses of melamine and its analogues may vary from one country to another.

Migration of melamine from melamine–formaldehyde plastic tableware products has been studied under controlled conditions using high temperatures and food-simulating solvents (e.g. acetic acid, ethanol, water). Reported levels of melamine migration can range considerably depending on the experimental conditions (Ishiwata, Inoue & Tanimura, 1986; Ishiwata et al., 1987; Bradley et al., 2005; Lund & Petersen, 2006). Additionally, recent melamine migration data from the Chinese Center for Disease Control and Prevention (unpublished data, 2008) and the Korea Food and Drug Administration (unpublished data, 2008) were also considered. It was agreed by the Expert Meeting that the concentration of melamine in foods from migration is likely to be less than 1 mg/kg (Table 2), given some of the harsh experimental conditions that were used.

Table 2. Melamine/cyanuric acid baseline sources and reported occurrence levels

Sources	MEL/ CYA	Reported level (mg/kg)	Additional notes	References
Migration from plastic tableware[a]	MEL	<1	Water, ethanol at 60 °C, 70 °C and 95 °C	Ishiwata, Inoue & Tanimura (1986); Bradley et al. (2005); Lund & Petersen (2006); Chinese Center for Disease Control and Prevention (unpublished data, 2008); Korea Food and Drug Administration (unpublished data, 2008)
		<1	3% acetic acid at 60 °C and 70 °C	
		0.18–42.9	4% acetic acid at 95 °C	Ishiwata, Inoue & Tanimura (1986)
		0.5–2.2	Coffee, orange juice, lemon juice, fermented milk at 95 °C	Ishiwata et al. (1987)
Cyromazine degradation	MEL	0.017–0.917	Residues in tomato, lettuce, celery from Japan	Japan Food Safety Commission (unpublished data, 2007)
		<1	Residues on vegetative part of bean	Karras et al. (2007)
		~10% of supervised trial median residue (STMR) in most crops		FAO (2007a,b)
		Concentration ≈ STMR in mushroom and offal		
Trichloro-melamine	MEL	0.14		USFDA (unpublished data, 2008)
Sodium dichloro-isocyanurate	CYA	1.6–3.2 mg/ml	Based on 1–2 mg/ml of free chlorine	WHO (2004)

CYA, cyanuric acid; MEL, melamine.

[a] Migration was analysed after assuming multiple conditions of exposure, which varied in duration.

Melamine is a metabolite of cyromazine, which can be used as a pesticide or veterinary drug. Residues of cyromazine and melamine have been detected on vegetable crops after spray application (Patakioutas et al., 2007). Data reported from Japan indicate that residual levels of melamine in tomato, lettuce and celery following agricultural trials were below 1 mg/kg (Japan Food Safety Commission, unpublished data, 2007). Karras et al. (2007) measured melamine residues in bean plants after applying cyromazine in solution to the bean roots; melamine residues in the vegetative part of the bean remained below 1 mg/kg. Cyromazine use as a pesticide was also evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 2007, where maximum residue limits (MRLs) were set for cyromazine in a number of crops and animal products (FAO, 2007a,b). JMPR (FAO, 2007a,b) and the USEPA (1999) have reported that melamine residues are generally ~10% of cyromazine residues, except in edible offal and mushrooms, where residues of melamine were of a similar magnitude to those of cyromazine. The European Union (EU) has set MRLs

for the use of cyromazine as a veterinary drug, but no data on residues of melamine as a result of this particular use were available (EU, 2001).

Trichloromelamine, which can decompose to melamine, is permitted for use in the USA in sanitizing solutions for food processing equipment and food contact articles (with the exception of milk containers or equipment). The USFDA (unpublished data, 2008) estimated a melamine dietary concentration of approximately 0.14 mg/kg based on the assumption that all sanitizers contain trichloromelamine.

Melamine can be degraded via deamination reactions to analogues such as ammeline, ammelide and cyanuric acid. Similar to melamine, cyanuric acid can occur as a degradation product of s-triazine pesticides. However, possible occurrence levels of cyanuric acid in food originating from these sources are currently unknown.

Trace levels of cyanuric acid can be present in food and water from the use of dichloroisocyanurate in drinking-water, swimming pools and water used in food manufacturing. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) estimated residues of cyanuric acid in water from the use of sodium dichloroisocyanurate as a disinfectant in water treatment (WHO, 2004). Using a conservative assumption that 1 mol of sodium dichloroisocyanurate would generate 1 mol of cyanuric acid, it was estimated that the cyanuric acid concentration would range from approximately 1.6 mg/l (equivalent to 1.0 mg/l of free chlorine) up to 3.2 mg/l (for a maximum dose of 2.0 mg/l of free chlorine). However, the review indicated that the use of sodium dichloroisocyanurate as a drinking-water disinfectant would be primarily for emergency situations.

Melamine and cyanuric acid may also enter the environment as a result of other legitimate widespread uses, such as the manufacturing of laminates, flame retardants, paints and fertilizer mixtures. However, no specific data on residual levels of melamine have been reported. At this time, any levels originating from these sources are expected to represent only a fraction of baseline levels to which humans are exposed. Industrial-scale uses, production and disposal of melamine may also lead to low levels of melamine in water effluents. Melamine monitoring data from rivers in Japan indicated levels ranging from 0.0001 to 0.0076 mg/l in water, from 0.01 to 0.4 mg/l in sediment and from 0.02 to 0.55 mg/l in fish (OECD, 1998). However, these data were considered to be insufficient to estimate possible levels in drinking-water or fish in general.

No information on residues of ammelide or ammelide from any source was provided for this Expert Meeting.

5.3 Melamine-contaminated milk incident in China in 2008

In 2008, high levels of melamine were detected in some infant formula and other liquid and powdered milk products originating from China. In many cases, analyses for cyanuric acid, ammeline and ammelide were not conducted. However, the contaminant profile appeared to consist primarily of melamine in this particular incident. China's General Administration of Quality Supervision, Inspection and Quarantine announced, in mid-September 2008, the results of an investigation into the extent of melamine contamination of dairy products (Chan, Griffiths & Chan, 2008; EFSA, 2008a; WHO, 2008a, 2008b). Of the 175 manufacturers of domestic powdered infant formula, 66 halted production, whereas 109 manufacturers had their products tested by China's General Administration of Quality Supervision, Inspection and Quarantine. Of 491 batches of products that were sampled, 69 batches produced by 22

companies contained detectable levels of melamine. Melamine levels in these products varied between 0.09 and 2563 mg/kg. In addition, tests conducted on liquid milk showed that 24 of 1202 batches contained melamine; the highest concentration reported was 8.6 mg/kg (Chan, Griffiths & Chan, 2008). More recent data generated by the Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, on the contamination of 111 Sanlu (the major manufacturer) infant formula samples indicated a mean melamine level of 1212 mg/kg, with individual samples ranging in concentration from <0.05 to 4700 mg/kg (Table 3). It is believed that melamine was added to raw ingredients to increase their apparent protein content, after these products had been diluted with water.

Table 3. Melamine concentrations in Sanlu infant formula samples[a]

	No. of samples	Mean (mg/kg)	Median (mg/kg)	90th percentile (mg/kg)	Maximum (mg/kg)	Range (mg/kg)
A	111	1212	1000	2600	4700	<0.05-4700
B	52	1674	1700	2880	4700	<0.05-4700

[a] A: all samples; B: samples collected from affected area in China. Limit of quantification 0.05 mg/kg.

5.4 National monitoring and surveillance data

The adulteration of milk and milk products with melamine in China has prompted investigations worldwide into the concentrations of melamine—and, in some cases, related analogues—in milk, milk ingredients and composited products containing milk-derived ingredients. In many cases, the primary focus has been on Chinese dairy products and other mixed foods containing dairy ingredients manufactured in or sourced from China. Some surveys have also focused on determining baseline levels of melamine in these types of products for imported and domestically produced items. Results from many countries were provided according to a limit of reporting for regulatory purposes, due to the setting of maximum limits for the presence of melamine and/or melamine and its analogues in dairy-based foods and/or foods containing milk ingredients, rather than as an analytical level of determination.

More recently, international media reports have raised concerns about melamine contamination of fruits and vegetables grown in China, fresh eggs, powdered and liquid egg products, non-dairy creamers, ammonium bicarbonate and animal feed originating from China, which may have been exported to other countries (FSANZ, 2008; HKCFS, 2008; INFOSAN, 2008; Kuo & Kang, 2008; Korea Food and Drug Administration, personal communication, 2008). As a result, testing of these types of products and products containing these ingredients was initiated.

5.4.1 Baseline levels

The form in which many national surveillance and monitoring data were supplied did not always allow for the separation of adulterated samples from those that were analysed for the purposes of determining baseline levels. Generally, data that were submitted to WHO by various countries for the current Expert Meeting, as well as information available on the web pages of international scientific bodies, indicate that the majority of samples analysed were below the limit of reporting and that baseline levels of melamine and related analogues are below 1 mg/kg.

For the purposes of determining baseline levels of melamine in foods, the Health Canada data sets on infant formula and milk- and soy-containing products (Health Canada, 2008a) were utilized for dietary exposure purposes. Health Canada analysed 80 infant formula products available on the retail market in order to determine background concentrations of melamine. Melamine was detected in 60 of 80 infant formula products, representing a 75% incidence of positive detections of low baseline levels of melamine. Concentrations of melamine ranged from 0.0043 to 0.346 mg/kg "as purchased". Concentrations of melamine in products "as consumed", after accounting for reconstitution factors in concentrated and powdered products, ranged from 0.000 53 to 0.0689 mg/kg. Samples were analysed using LC-MS/MS to quantify melamine at a limit of detection of 0.004 mg/kg. Similar data on baseline levels of melamine in infant formula were provided to WHO by the USFDA, the Bureau of Food and Drug Analysis, Department of Health, of Taiwan, China, the National Taiwan University, Food Standards Australia New Zealand and the New Zealand Food Safety Authority (unpublished data, 2008). Although the levels or absence of melamine in these products was consistent with the Health Canada data set, the Health Canada data set utilized a more sensitive limit of detection, allowing for the determination of melamine at lower levels.

An additional Health Canada survey (Health Canada, 2008b) determined the presence of melamine in 242 samples of domestic milk, domestic or imported finished foods containing milk, and milk- or soy-derived ingredients, as well as composite foods containing milk ingredients available in Asian markets. Positive results for melamine in milk- or milk ingredient–containing products, other than infant formula, were generally low and ranged from 0.004 35 to 0.282 mg/kg. This data set was used for estimating dietary exposures from foods other than infant formula, owing to the sensitivity of the method utilized in this survey. The Expert Meeting determined that this data set was the best available to represent baseline levels of melamine in foods other than infant formula.

5.4.2 Contaminated foods containing milk and milk-derived ingredients other than infant formula

For the determination of the dietary exposure (discussed in section 6) resulting from the consumption of adulterated products, the Expert Meeting utilized the data compiled by WHO on confirmed and reported contaminated food products, as distributed to national food safety authorities through INFOSAN (http://www.who.int/foodsafety/fs_management/infosan/en/index.html), and the list of contaminated products from the European Commission's Rapid Alert System for Food and Feed (http://ec.europa.eu/food/food/rapidalert/index_en.htm). In many cases, countries appear to have submitted only positive concentrations that were above established maximum levels for melamine and related analogues in food, whereas other countries submitted data on any positive determinations they may have encountered, regardless of whether they were above maximum limits. Concentrations of melamine in samples testing positive for melamine are shown in Table 4.

More recently, elevated levels of melamine have been found in non-dairy creamers from two lots (745.2–20 700 mg/kg) in Taiwan, China, and in ammonium bicarbonate (70–2470 mg/kg) in Malaysia, Taiwan, China, and the Republic of Korea (BERNAMA, 2008; Kuo & Kang, 2008; Korea Food and Drug Administration, personal communication, 2008). The source of the contamination of these products is not known at this time.

Table 4. Melamine concentrations in food and feed samples testing positive for melamine

Food item	Melamine concentration (mg/kg)
Biscuits, cakes and confectionery	0.6–945.86
Liquid milk and yoghurt products	0.5–648
Frozen desserts	39–60.8
Powdered milk and cereal products	0.38–1143
Processed foodstuff	0.6–41
Food processing ingredients	1.5–6694
Animal feed	116.2–410

5.5 Melamine and related analogues in feed

Melamine may be present in animal feed at baseline concentrations owing to the use of triazine pesticides such as cyromazine on crops (Root, Hongtrakul & Dauterman, 1996; Weintraub, 2001; Sancho et al., 2005; Karras et al., 2007; Patakioutas et al., 2007; USEPA, 2007) or the use of cyromazine as a veterinary drug (EFSA, 2007; Karras et al., 2007) or as a result of fertilizer–urea mixture applications. Codex Alimentarius Commission MRLs for cyromazine are 0.05 mg/kg for poultry meat and sheep meat and 0.01 mg/kg for milk (http://www.codexalimentarius.net/mrls/pestdes/jsp/pest_q-e.jsp). Several countries have established MRLs for cyromazine in food of animal origin, including meat, milk and eggs (EFSA, 2007). Also, cyanuric acid may be present at baseline concentrations in ruminant feed containing biuret in the USA, where biuret may contain up to 30% cyanuric acid (USFDA, 2003b).

5.5.1 Misuse or adulteration

Melamine or cyanuric acid may be present in animal feed at concentrations above baseline as a result of misuse or adulteration. However, occurrence data that relate to the adulteration of feed items with melamine are limited. For example, melamine was detected in pelleted fish and shrimp feed owing to the use of melamine as a pelletizing agent (JAVMA, 2007; USFDA web site on recalls, market withdrawals and safety alerts: http://www.fda.gov/opacom/7alerts.HTML). Andersen et al. (2008) and Karbiwnyk et al. (2008) found melamine in commercial salmon, trout and shrimp feed at concentrations of 6.7, 0.5 and 170 mg/kg, respectively. INFOSAN (2008), as well as others, has reported that the presence and/or addition of melamine to animal feed originating from China may be widespread (Cattaneo & Ceriani, 1988; Barboza & Barrionuevo, 2007). The European Commission's Rapid Alert System for Food and Feed recently reported the adulteration of animal feed (organic soy expeller) originating from China in France, Germany and the United Kingdom. Concentrations were in the range of 1.6–410 mg/kg (INFOSAN, 2008; RASFF, 2008; FAO, personal communication, 2008).

5.5.2 Carry-over

Carry-over of melamine from animal feed into food of animal origin has been demonstrated in swine and fish.

Limited data are available on the carry-over of melamine from feed into foods of animal origin, based on available information from the 2007 pet food incident. In a study in which pet food scraps were incorporated into animal feed and fed to hogs at

69–74 mg/kg (B. Puschner, personal communication, 2008), concentrations of melamine in the loin and ham were 9–12 µg/kg in three hogs slaughtered 1 day after feeding and <10 µg/kg in two additional hogs slaughtered 7 days after feeding. A further two hogs slaughtered 14 days after exposure were found to be negative for melamine in loin and ham samples (USFDA, 2007). Melamine was not detected (limit of detection 50 µg/kg) in the meat of any of the six chickens analysed that were inadvertently fed contaminated feed (USFDA, 2007). Studies conducted by Andersen et al. (2008) and Karbiwnyk et al. (2008), in which it was determined that commercial salmon and trout feed contained melamine at concentrations of 6.7 and 0.5 mg/kg, respectively, found residues of 40–120 µg/kg in the muscle tissue of non-dosed (control) trout and salmon. In addition, a survey of 105 market-ready fish found detectable levels of melamine in 33 samples above the limit of detection of 3.2 µg/kg. Ten of these samples had concentrations greater than 50 µg/kg, ranging from 51 to 237 µg/kg (Andersen et al., 2008). Other data reporting the carry-over of melamine from feed into the tissues or products of animal origin were not available.

The National Measurement Institute of Australia reported melamine in two samples of egg albumin at 11 and 12 mg/kg (Food Standards Australia New Zealand, unpublished data, 2008). Melamine was found in dry whole eggs in Japan at concentrations ranging from 2.8 to 4.6 mg/kg (INFOSAN, 2008; Japan Food Safety Commission, unpublished data, 2008) and in an egg product from the USA at 1.1 mg/kg (USFDA, unpublished data, 2008). Egg powders in China were found to contain melamine at concentrations ranging from 0.1 to 4 mg/kg, as reported by INFOSAN (2008). Positive results for the presence of melamine were found in egg powder (whole egg powder: 0.84–4.00 mg/kg; egg white powder: 1.30–2.50 mg/kg; egg yolk powder: 0.11 mg/kg) and in liquid egg yolk (0.48 mg/kg) products available for sale in the Republic of Korea; however, none of the 1202 meat samples were found to contain melamine above the limit of quantification of 0.1 mg/kg (National Veterinary Research and Quarantine Service, Ministry of Food, Agriculture, Forestry and Fisheries, Republic of Korea, unpublished data, 2008). Melamine was also found in Japan in fried chicken at 1.6 mg/kg and in frozen takoyaki (octopus) at concentrations ranging from 0.6 to 1.6 mg/kg (INFOSAN, 2008; Japan Food Safety Commission, unpublished data, 2008). In addition, fresh eggs from China were found to contain melamine at concentrations ranging from 2.9 to 4.7 mg/kg (INFOSAN, 2008). It is thought that the presence of melamine in these samples may be a result of carry-over from adulterated animal feed. A recent report from South Africa (Reyers, 2008) suggests that the presence of melamine in raw milk may be a result of carry-over of melamine from melamine-contaminated raw materials used in animal feed.

6. EXPOSURE ESTIMATES FOR MELAMINE AND ITS ANALOGUES

In principle, the Expert Meeting made the most realistic estimate of exposure using the available data for each source. However, owing to the limitations of the data, these estimates were generally conservative and in some cases highly conservative. The level of conservatism and uncertainties have been documented. The dietary exposure assessments for baseline levels are expressed as **μg**/kg body weight per day. The dietary exposure assessments resulting from adulteration or misuse are expressed as **mg**/kg body weight per day.

A number of national authorities have undertaken a calculation of the concentration in the contaminated food at which their respective tolerable daily intake (TDI) would be reached for melamine or a predictive level of exposure based on theoretical adulteration levels in food.

6.1 Description of submitted or available data

6.1.1 Levels of melamine and its analogues in food

Two lists of levels were identified from the available occurrence data. Low-level concentrations, which were unlikely to be present as a result of adulteration or misuse, were used in the baseline-level exposure assessment. Other relevant occurrence data were used to assess the dietary exposure resulting from adulteration or misuse.

6.1.2 Food consumption data

Where dietary exposure assessments have been undertaken de novo, relevant food consumption data have been utilized. Dietary exposure assessments have been done for both processed foods and raw agricultural commodities. However, there are no food consumption data that describe foods actually consumed by populations at a global or regional level. The Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) consumption cluster diets describe the amount of raw food commodities available for consumption in 13 regions (WHO, 2009). These data have been used for the estimation of dietary exposure to melamine residues as a result of the use of cyromazine. For dietary exposures from the consumption of infant formula, estimates were based on food consumption data for specific countries. For other processed foods, estimates of dietary exposure used the Concise European Food Consumption Database, which contains food consumption data for 17 European countries (EFSA, 2008b).

6.1.3 Uncertainty regarding overall exposure to melamine and its analogues

The dietary exposure assessments conducted de novo by the Expert Meeting for melamine from all sources of exposure other than infant formula are very conservative. The dietary exposure assessment for cyanuric acid in drinking-water is also very conservative. In addition, there is a high level of uncertainty associated with the exposure estimates due to the limited number of data available.

6.2 Exposure to melamine and its analogues resulting from baseline levels

Dietary exposure to cyanuric acid, ammeline and ammelide in food could not be estimated because very limited concentration data were available. Only cyanuric acid exposure from drinking-water could be estimated, based on use levels rather than measured concentrations.

6.2.1 Infant formula

The Expert Meeting agreed that the Health Canada infant formula survey data (Health Canada, 2008a) represented the most appropriate occurrence data on which to undertake a baseline dietary exposure assessment, owing to the sensitivity of the analytical method used. Other national survey data on infant formula from the USA (USFDA, unpublished data, 2008), Taiwan, China (Chen & Kang, 2008), New Zealand (J. Reeve, personal communication, 2008) and Australia (J. Baines & L. Laajoki, personal communication, 2008) were available; results were consistent with the findings of the Health Canada survey. Dietary exposure assessments were undertaken for different infant age groups using published food consumption data for each group (Institut National de Santé Publique du Québec, 2001) or for premature infants using a Health Canada estimate of consumption (Health Canada, 2008a). The dietary exposure assessment used mean concentration and consumption data, and "non-detects" were set to one half the detection limit of 4 µg/kg. Estimated dietary exposures to melamine were 0.54–1.6 µg/kg body weight per day.

6.2.2 Foods other than infant formula

Estimates of baseline dietary exposure to melamine were undertaken for 17 countries in Europe, a region where processed foods form a significant part of the diet. Mean food consumption amounts for the whole population for foods of interest were taken from the Concise European Food Consumption Database, which reports food consumption amounts across 14 broad food categories (EFSA, 2008b). Melamine concentrations were derived from Health Canada data for foods available on the Canadian market (Health Canada, 2008b).

Where data were available for a single food only, the mean level was taken to represent all foods in the category, with non-detects being assigned the limit of detection of 4 µg/kg. If more than one food had been analysed per food category, the highest mean baseline level was taken (data for cereal mixed dishes, sugar products including chocolate, coffee, tea, milk-based drinks, dairy-based products and cheese food categories).

Estimated dietary exposure to melamine at baseline ranged from 0.03 to 0.12 µg/kg body weight per day across the 17 European countries, assuming it was present in all food groups included with a "positive result" for a food in the category and an average adult body weight of 60 kg. This is a conservative estimate, as the highest upper-bound mean baseline result for any one food group was used for all foods in that food category.

6.2.3 Chlorine-containing disinfectants

6.2.3.1 Trichloromelamine

Trichloromelamine, approved for use as a disinfectant in the USA, decomposes to melamine. Based on the very conservative assumptions that all

disinfectants used in food processing contain trichloromelamine and that 3 kg of food are consumed per day for a 60-kg adult, dietary exposure to melamine was estimated to be 7 µg/kg body weight per day.

6.2.3.2 Sodium dichloroisocyanurate

The disinfection of drinking-water with dichloroisocyanurate may lead to residues of cyanuric acid. The 61st meeting of JECFA estimated dietary exposure of 70 µg/kg body weight per day for cyanuric acid (WHO, 2004). However, this estimate is very conservative, as it assumes that 1 mol of sodium dichloroisocyanurate results ultimately in 1 mol of cyanuric acid and uses the maximum application rate of sodium dichloroisocyanurate (3.2 mg/l, equivalent to 2.0 mg/l of free chlorine).

6.2.4 Migration from food contact materials

6.2.4.1 Migration from melamine-containing plastics

Melamine is a raw material in the production of some plastic products used for serving food. Low-level migration of melamine into food has been reported (e.g. Korea Food and Drug Administration, 2007; Chinese Center for Disease Control and Prevention, unpublished data, 2008). The Expert Meeting reviewed the available migration data and agreed that concentrations in food are likely to be <1 mg/kg (see section 5.2). Although there are some limited data on concentrations above 1 mg/kg, it was noted that the assays used harsh migration conditions (typically 3% acetic acid, 70 °C for 2 h) that would not be encountered in practice. Using a concentration of 1 mg/kg and applying an assumption that the migrant may be present in 25% of the diet[1] (i.e. 750 g/person per day for an adult), the Expert Meeting made a conservative estimate of dietary exposure to melamine for 60-kg adults of 13 µg/kg body weight per day.

6.2.4.2 Migration of melamine from the use of melamine-containing adhesives in food contact materials

The USFDA (unpublished data, 2008) reported that melamine is regulated as a component of an adhesive used in food contact materials. The USFDA estimated the dietary exposure to melamine from its use in adhesives presuming "virtually nil" migration. Dietary exposure to melamine of <0.35 µg/kg body weight per day was calculated based on an adult of 60-kg body weight. A similar calculation was undertaken for the use of melamine in paper and paperboard, yielding a melamine dietary exposure of 0.0019 µg/kg body weight per day.

6.2.4.3 Migration from melamine used in melamine–formaldehyde resins

The USFDA (unpublished data, 2008) reported that melamine is incorporated into melamine–formaldehyde resins used for the coating of food tins. As the melamine is incorporated in the resin, only residual melamine would be available for migration. No data were available that would allow the estimation of dietary exposure to melamine as a result of melamine–formaldehyde resins in contact with food.

---

[1] The USFDA commonly uses a model diet that assumes 3 kg total food consumption, of which 1.5 kg is solid foods.

6.2.5 *Residues arising from the use of cyromazine as a pesticide and as a veterinary drug*

The 38th meeting of JMPR assessed residues of cyromazine in various commodities following use as a pesticide in a range of crops and in animal feed (FAO, 2007a,b). Melamine is a metabolite of cyromazine in plants, mammals, poultry and ruminants. The 2007 meeting agreed that for practical purposes, the definition of residues for plants and animal commodities should be parent cyromazine only.

For the dietary exposure assessment, melamine concentrations were derived from the supervised trial median residue (STMR) levels for cyromazine (agricultural trial data; FAO, 2007a,b) for all products for which a MRL for cyromozine was estimated by the 2007 JMPR. Melamine residue concentrations were assumed to be 10% of the cyromazine residue levels[1] for the purpose of the dietary exposure assessment. However, JMPR data indicate that for edible offal (mammalian meat offal and poultry offal) and mushrooms, levels of melamine were of a similar magnitude to those of the parent cyromazine. For these latter commodities, it was assumed that melamine residue concentrations were at the STMR levels of 0.01 and 2.2 mg/kg, respectively.

Food consumption amounts for the products likely to contain melamine from the legitimate use of cyromazine as a pesticide for 13 different regions in the world were derived from the GEMS/Food consumption cluster diets (WHO, 2009). Estimated dietary exposure to melamine from use of cyromazine as a pesticide ranged from 0.04 µg/kg body weight per day (diet A) to 0.27 µg/kg body weight per day (diets E and M). It was assumed that residues were present in all commodities for which a MRL was estimated by the 2007 JMPR in all 13 regions. An adult body weight of 60 kg was also used. This is a conservative estimate, as it assumes that all crops have been treated, that cyromazine has been applied at the maximum use pattern[2] and that it will be used in all regions.

Cyromazine is also used as a veterinary drug. However, no data on residue levels of cyromazine or its metabolites were available. Levels arising from the veterinary use of cyromazine were therefore not included in the dietary exposure assessment.

6.2.6 *Addition to animal feed*

No data were available on the baseline levels of melamine in animal feed.

Cyanuric acid may be present in feed in the USA as a result of the addition of food-grade biuret (≤30% cyanuric acid). However, a dietary exposure assessment has not been carried out because there were no data on the transfer of cyanuric acid into animal food products as a result of feed consumption.

6.2.7 *Other potential sources of melamine exposure*

The Expert Meeting received limited information suggesting that melamine may be added to fertilizer to control the rate of release of nitrogen into the soil. No supporting data were available to the Expert Meeting.

The Expert Meeting noted that exposure to melamine may also result from the use of melamine in paints. Similarly, the Expert Meeting noted that oral ingestion of cyanuric acid may result from the use of sodium dichloroisocyanurate in swimming pools. Exposure assessments were not undertaken for these other potential sources of

---

[1] The Expert Meeting recognized that the 10% value was a rough approximation of the ratio between cyromazine and melamine residues. For some residue trials, melamine levels were less than 10% of the cyromazine levels, and for others, they were greater than 10%.

[2] Commonly referred to as "Good Agricultural Practice".

exposure to melamine and cyanuric acid because of the lack of relevant data available to the Expert Meeting.

Table 5 summarizes the dietary exposure estimates for melamine and cyanuric acid from various sources at baseline levels.

Table 5. Summary of estimates of exposure to melamine at baseline levels from various sources

Category	Estimated daily exposure (µg/kg body weight)[a]	Comment
Infant formula	0.54–1.6	Mean exposure
Other foods	0.03–0.12	Adults, mean exposure
Disinfection in food processing	7	Adults, very conservative estimate
Migration from melamine-containing plastics	13	Adults, conservative estimate
Migration from melamine-containing adhesives	<0.35	Adults, conservative estimate
Residues arising from cyromazine use	0.04–0.27	Adults, conservative estimate

[a] The Expert Meeting considered that it was not appropriate to sum the dietary exposure assessments from different sources, as 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.

6.3 Exposure to melamine and its analogues resulting from adulteration or misuse

6.3.1 Infant formula

In a Chinese dietary exposure assessment specific to adulterated Sanlu infant formula products, four age groups of infants and young children (3, 6, 12 and 24 months) were chosen. The maximum amount of infant formula consumption was used based on the recommended usage level on the package label of Sanlu infant formula and other brands. In estimating dietary exposure for the four groups, the body weight and infant formula consumption values used were in the ranges 5.5–14 kg and 120–150 g, respectively. The concentration data from 111 samples of Sanlu infant formula were used, as summarized in Table 3 (Chinese Center for Disease Prevention and Control, unpublished data, 2008). The calculated estimates of dietary exposure are summarized in Table 6.

Table 6. Estimated dietary exposure to melamine from Sanlu infant formula (111 samples)

| Age (months) | Melamine dietary exposure estimates (mg/kg body weight per day) | | | |
	Mean concentration (1212 mg/kg)	Median concentration (1000 mg/kg)	90th-percentile concentration (2600 mg/kg)	Maximum concentration (4700 mg/kg)
3	28.4	23.4	61.0	110.2
6	26.0	21.4	55.7	100.7
12	18.2	15.0	39.0	70.5
24	10.4	8.6	22.3	40.3

Based on the median melamine concentration (1000 mg/kg), the melamine dietary exposures for infants of 3, 6, 12 and 24 months were 23.4, 21.4, 15.0 and 8.6 mg/kg body weight per day, respectively. The researchers emphasized that the samples collected were not necessarily representative, that melamine concentrations in the adulterated Sanlu infant formula varied and that infants may not have consumed Sanlu product all the time.

6.3.2 *Foods other than infant formula*

Adulteration has been recently documented in milk and milk powder, cereal products and ammonium bicarbonate. Foods that may contain milk (powder) include dairy foods such as ice cream and yoghurt, meal replacements containing dairy ingredients, biscuits, cakes, chocolates, ice confections and milk-based drinks. Some of these foods also contain cereal. Although occurrence data were available for ammonium bicarbonate, eggs and egg powder, it was not possible to include this information in the model, other than to assume that where this additive had been used as a raising agent in products such as biscuits and cakes or where egg products had been added, the contribution to overall melamine levels in that product would have been accounted for in the reported analytical results from the INFOSAN reports of contaminants (http://www.who.int/foodsafety/fs_management/infosan/en/index.html) and the EU Rapid Alert System for Food and Feed (http://ec.europa.eu/food/food/rapidalert/index_en.htm).

For the purpose of estimating dietary exposure to melamine for foods containing dairy products from China, estimates were undertaken for the same 17 countries in Europe for which baseline estimates were previously given (see section 6.2.2), using the Concise European Food Consumption Database for adults (EFSA, 2008b). Melamine concentrations for each relevant food category were derived from the maximum melamine concentrations from INFOSAN (http://www.who.int/foodsafety/fs_management/infosan/en/index.html) and other country data. It was assumed that 10% of each food category for which there were analytical results (data for cereal mixed dishes, other cereals, sugar products including chocolate, coffee, tea and milk-based drinks food categories) may have contained milk powder from China and that coffee comprised 50% of the tea and coffee category. In cases where more than one food had been analysed per food category, the highest reported melamine level was taken.

Estimated dietary exposures to melamine from foods containing adulterated milk powder were 0.16–0.7 mg/kg body weight per day, assuming that it was present in all food groups with the highest reported result for a food in that group and an average adult body weight of 60 kg. This is a very conservative estimate, as in reality not all of the food in each food category is likely to contain milk powder, and less than 10% of foods are likely to contain adulterated Chinese milk powder. In addition, actual melamine levels are likely to be lower than the maximum reported result for any one food category.

Prior to the availability of testing results, the European Food Safety Authority (EFSA) published a theoretical worst-case estimate of melamine dietary exposure for high consumers of biscuits and confectionery, assuming a range of melamine concentrations in milk powder and various proportions of milk powder in biscuits and confectionery (EFSA, 2008a). Estimates of potential dietary exposure to melamine from biscuits ranged from 0.0002 to 0.3 mg/kg body weight per day for a 60-kg adult and from 0.0006 to 0.9 mg/kg body weight per day for a 20-kg child. Estimates of potential dietary exposure to melamine from confectionery ranged from 0.0007 to

0.45 mg/kg body weight per day for a 60-kg adult and from 0.002 to 1.35 mg/kg body weight per day for a 20-kg child.

6.3.3 Addition to animal feed

No farm animal feeding studies were available that would allow a quantitative assessment of the carry-over of melamine into foods from animal feed to be carried out. The Expert Meeting therefore did not perform a dietary exposure assessment relating to the recent reports of adulteration of animal feed.

7. EPIDEMIOLOGICAL AND TOXICOLOGICAL DATA

7.1 Biochemical aspects

7.1.1 Melamine

In rats, melamine is rapidly absorbed from the intestine and attains maximal plasma concentrations in 1 h following a single oral dose (Mast et al., 1983; Sugita, Ishiwata & Maekawa, 1991). The plasma half-life is approximately 2.7 h. Melamine is eliminated essentially unchanged by the kidney (Worzalla et al., 1974; Mast et al., 1983). In the pig, the melamine plasma half-life is approximately 4 h, with a clearance of 0.11 l/h per kilogram and a volume of distribution of 0.61 l/kg (Baynes et al., 2008).

Limited information is available on other species. Melamine has been detected in urine of dogs (Lipschitz & Stokey, 1945) and in urine of cats sickened during the pet food episode of 2007 (Brown et al., 2007). In a case-report, melamine has been found to be excreted in the milk of cows exposed to melamine-contaminated high-protein concentrate (Reyers, 2008).

7.1.2 Cyanuric acid and other structural analogues

In rats, cyanuric acid is absorbed rapidly and eliminated unchanged in the urine, with a half-life of approximately 1–2.5 h, depending on the dosage administered. Cyanuric acid may be present in the faeces if administered in high doses (500 mg/kg body weight). In dogs, the half-life is 1.5–2 h, and elimination is also via the kidneys. No metabolites of cyanuric acid were detected in the urine or faeces (Barbee et al., 1984; Hammond et al., 1986).

In humans, absorption and excretion of cyanuric acid have been studied in long-distance swimmers exposed by swimming in pools disinfected with chlorinated isocyanurates and in two volunteers given an unspecified solution of cyanuric acid orally. More than 98% of the administered dose was recovered unchanged in urine after 24 h. The half-life of excretion was about 3 h (Allen, Briggle & Pfaffenberger, 1982).

No information was available for other structural analogues.

7.1.3 Melamine plus cyanuric acid

Limited information is available regarding the absorption and distribution of melamine simultaneously administered with cyanuric acid (as separate chemicals, not as the melamine–cyanurate complex). In pet dogs and cats during the 2007 episode, melamine was identified in urine, and crystals composed of melamine–cyanurate were present in kidneys. Melamine and cyanuric acid were also detected in the kidneys of cats dosed with both melamine and cyanuric acid together (Puschner et al., 2007). Similarly, muscles and kidneys from fish orally given both melamine and cyanuric acid contained residues, indicating that both compounds are absorbed and subsequently excreted. In fish, a large amount of white precipitate containing crystals was also present in the faeces of high-dose animals (400 mg/kg of each compound) (Reimschuessel et al., 2008).

The absorption and excretion of the melamine–cyanurate complex may be quite different from the absorption and excretion of these compounds individually. Preliminary data in fish show very limited absorption of the complex, even with high

doses (400 mg/kg for 3 days) (R. Reimschuessel, unpublished data, 2008). The fate of the complex in mammals is currently unknown. There may be limited dissociation of the complex in an acidic stomach, but there are no studies examining absorption of the complex.

7.2   Clinical and epidemiological findings

According to a report from the Chinese Ministry of Health[1], since 1 December 2008, 22 384 000 examinations have been conducted in infants with suspected melamine exposure. As of 27 November 2008, 294 000 infants had been diagnosed with urinary tract abnormalities. The number of hospitalized infants is 51 900, with 51 039 infants discharged and 861 still in the hospital.

7.2.1 Clinical cases

The case-studies discussed below represent a subsample of clinical cases reported during the outbreak.

Case definition: An infant exposed to melamine-contaminated infant formula with confirmed presence of calculi in the kidney, ureter or bladder with or without clinical symptoms.

Available data:
1. Beijing Children's Hospital: A total of 32 530 children were examined by ultrasound at the Beijing Children's Hospital from 12 September to 17 November 2008. Of all screened children, 2.9% had stones, of which 4.2% were hospitalized. The average time for hospitalization was 13 days. Melamine concentrations were determined in some of the infant formula consumed by the affected children (Y. Shen, personal communication, 2008).

2. Beijing Children's Hospital and Xuzhou Hospital: Thirty-four children were diagnosed with calculi at the Beijing Children's Hospital (25 children) and Xuzhou Hospital (9 children) between January and September 2008. All 34 children had acute renal failure and were hospitalized. The average time for hospitalization was 16 days. All 34 children had consumed Sanlu infant formula. However, none of the infant formula consumed by these children was available for analysis. For 25 children (ages 6–36 months; median 8.5 months), the duration of Sanlu infant formula consumption was known and was between 15 days and 13 months (median 8 months). The infant who consumed the infant formula for 15 days was an 8-month-old female who was breastfed prior to being given Sanlu infant formula. The female had multiple stones in both kidneys and in the ureter where it attached to the bladder. The serum potassium was 5.57 mmol/l, blood urea nitrogen was 24.7 mmol/l and creatinine was 575.9 μmol/l. The girl was treated for 5 days with peritoneal dialysis and intravenous sodium bicarbonate. The stones were passed, and the girl recovered.

3. Other information: After China announced the melamine contamination of infant formula on 10 September 2008, the deaths of 11 infants were investigated further. Six

---

[1] Ministry of Health of the People's Republic of China (2008). Ministry of Health official website accessed 1 December 2008,
   http://www.moh.gov.ch/publicfiles/business/htmlfiles/mohbgt/s3582/200812/38386.htm

of the children had known exposure to contaminated infant formula, and medical records showed that stones were present. The reason for lethal outcome was most likely the lack or delay of treatment. Diagnostic investigations to confirm the presence of melamine in stones or tissues were not done for any of the six children. Based on medical histories and reviews of medical records, the deaths of the other five infants were considered unrelated to melamine exposure.

Clinical presentation: Clinical signs of symptomatic children included crying, vomiting, fever, haematuria, dysuria, oliguria, anuria, high blood pressure, oedema and pain in kidney areas. However, most children with stones did not show clinical signs.

Diagnostic work-up: Children with known exposure to contaminated milk products underwent a physical examination and were examined by ultrasound. Clinical chemistry results, including blood urea nitrogen and creatinine, indicated no changes, with the exception of increases in calcium in a limited number of patients. Blood was identified in urine of patients with blockage or who had passed stones. No other uric acid abnormalities were identified. White blood cell counts were increased in patients with infection. Ultrasound findings included sand-like calculi in the renal pelvis, ureter and/or bladder. The largest calculus was 1.9 cm in diameter. Most calculi were smaller than 0.6 cm in diameter. Some calculi formed clusters. When there was obstruction in the ureter, the renal pelvis and calices were enlarged. Ultrasound can detect calculi that are at least 1 mm in diameter.

Analysis of calculi: Calculi of hospitalized patients were analysed by LC-MS and GC-MS and contained uric acid and melamine. The ratio of uric acid to melamine (on a molar basis) ranged from 1.2:1 to 2.1:1 in 15 calculi analysed. Cyanuric acid was not detected in any of the calculi (Chinese Center for Disease Control and Prevention, unpublished data, 2008).

Treatment: Exposure to contaminated milk was stopped immediately. Children with stones were orally administered water and sodium bicarbonate or sodium citrate.

Children with clinical signs of illness, known obstruction or hydronephrosis were hospitalized. These children received the following treatment:

- Intravenous sodium chloride with dextrose and sodium bicarbonate or sodium citrate. Urine was alkalinized to reach a pH of 6.5–7.0. The urine pH of sick children prior to treatment was 5.5–6.0.
- Children with acute renal failure and oliguria for at least 2 days or anuria for at least 1 day were dialysed until clinical chemistry parameters returned to reference ranges.
- Surgical intervention with removal of stones was performed in patients with hydronephrosis, in patients where prior medical treatment was ineffective and in anuric patients when dialysis was ineffective or unavailable.

Efficiency of treatment was assessed by reduction in stone size over time and passage of stones. There was indication that sodium citrate may have been more effective than sodium bicarbonate in reducing stone size.

Follow-up of outpatients from the Beijing Children's Hospital, 12 September to 17 November survey: Of the 945 children with stones, 200 were examined with

ultrasound 1 month after initial evaluation. At that time, the majority of children still had stones, but most stones were smaller than 0.6 cm in diameter. One month later, 80% of the children who had stones smaller than 0.6 cm in diameter at the 1-month follow-up had passed the stones. Children with persistent stones continue to be evaluated monthly.

7.2.2 Cross-sectional study

Case definition: An infant exposed to infant formula with ultrasound confirmation of calculi in the kidney, ureter or bladder of greater than 2 mm in diameter.

Available data:
1. Liangzhou District, Wuwei Prefecture, Gansu Province: This study examined 2085 children born after 1 September 2005 in Gansu Province. Stones were identified in 348 children (17%) by ultrasound. Of these 348 children, 216 were male and 132 were female. Clinical signs were reported for 25% of infants with stones. Clinical signs, in order of prevalence, were as follows: crying while urinating, dysuria, presence of sand-like material in urine, oliguria, anuria and haematuria. In children with infection, fever, vomiting and coughing were also reported.

Children included in this study consumed 15 different brands of formula. Among the 2085 children evaluated, 851 children consumed Sanlu milk powder only, of which 23% (192) had urinary tract stones when examined by ultrasound. Serum chemistry parameters, including blood urea nitrogen, creatinine and uric acid, were not altered in children with stones compared with children without stones consuming Sanlu milk. No crystals were identified from urinalysis. Urinary tract stones were found in children who had not consumed Sanlu milk for up to 24 months prior to ultrasound examination. Factors increasing the risk of stone formation in children included the amount and the duration of consumption of Sanlu milk powder and young age (Tables 7 and 8).

Forty Sanlu milk powders collected in children's homes were analysed, and 93% contained melamine, whereas 73% contained cyanuric acid. The estimated melamine concentrations ranged from 150 to 4700 mg/kg (median 1900 mg/kg), and the cyanuric acid concentrations ranged from 0.4 to 6.3 mg/kg (median 1.2 mg/kg).

Table 7. Age at first consumption of Sanlu milk and incidence of stones

Age (months)	Number of examined infants (% of total)	Infants with stones (% of same-age infants examined)
<6	456 (54)	116 (25)
6–12	273 (32)	59 (22)
12–18	103 (12)	15 (15)
18–24	12 (1)	1 (8)
24–30	7 (1)	1 (14)
>30	0 (0)	0 (0)
Total	851 (100)	192 (23)

Table 8. Duration of Sanlu milk consumption and incidence of stones

Duration (months)	Number of examined infants (% of total)	Infants with stones (% of same-duration infants)
<6	270 (32)	42 (16)
6–12	193 (23)	52 (27)
12–18	190 (22)	44 (23)
18–24	131 (15)	33 (25)
24–30	61 (7)	20 (33)
>30	6 (1)	1 (17)
Total	851 (100)	192 (23)

It is important to note that although these data provide very important information, dose–response information cannot be generated. There was wide variation in the concentrations of melamine in infant formula between brands and within the same brand; in many cases, the concentrations were not determined; the exact amount of consumption could not be determined; and many children were exposed to different brands and for various times.

7.2.3 Screening studies

Case definition: An infant suspected to have consumed contaminated infant formula.

Available data:
1. Taipei Hospital, Taiwan, China: Between 24 September and 23 October 2008, children whose parents were concerned that they may have consumed melamine-contaminated dairy products were investigated at the Taipei Hospital of the Department of Health. A total of 999 children were included in this study and divided into three groups: a high-exposure group (defined as those children who consumed China-brand dairy products, such as Sanlu, Mengniu or Yili, with melamine levels above 2.5 mg/kg); a low-exposure group (defined as those children who consumed other brands of contaminated milk, such as Klim or Neslac imported from China, with melamine levels between 0.05 and 2.5 mg/kg); and a control group (defined as those children who consumed milk without detected melamine levels, such as Taiwan-brand infant formula, with melamine levels below 0.05 mg/kg). The following diagnostic parameters were established for all 999 children: clinical presentation, urinalysis, urine calcium, creatinine and renal ultrasonography. Seven children had nephrolithiasis. Testing of milk powder confirmed the presence of melamine in six of the seven cases.

2. Hong Kong Special Administrative Region (SAR), China: As part of Hong Kong SAR's screening programme in response to melamine contamination of milk and milk products, 45 000 children aged 12 years or younger were assessed at clinics. Urinary tract stones were identified by ultrasound in 10 children, ranging in age from 22 months to 10 years. Four children showed clinical signs, whereas the other six were asymptomatic.

7.2.4 Consequence of melamine exposure

The primary effect of consuming high doses of melamine alone has repeatedly been seen to be stone formation. This is true for both animal experiments and the infant formula incident (Ogasawara et al., 1995; Cremonezzi et al., 2001; Ministry of

Health of the People's Republic of China, 2008). In both animals and humans, the stones are composed primarily of uric acid and melamine. Since melamine's structure allows for hydrogen bonding with uric acid, co-precipitation is not unexpected. In the case of rats, the ratio is 1:1; in the case of humans, based on limited data, the ratio was 1.2:1 to 2.1:1. Perhaps the higher concentrations of uric acid found in humans, and specifically neonates, contribute to the higher ratio of uric acid in the stones.

7.2.5 Special considerations of infant physiology

It is important to examine aspects of infant physiology that could make them more susceptible to melamine-associated renal damage. The renal stones formed in infants ingesting melamine-contaminated formula were primarily composed of uric acid and melamine. Normal uric acid concentrations in infants are higher than those of older children or adults (Table 9) (Fathallah-Shaykh & Neiberger, 2008).

Table 9. Concentrations of uric acid in serum and urine of neonates, children and adults

	Uric acid concentration (mg/dl)	
	Serum	Urine
Neonates (29–33 weeks)	7.7	4.8
Neonates (34–40 weeks)	5.2–6	1.7–2.8
Children (3–9 years)	3.4–3.6	0.34
Children (10–14 years)	4	0.34
Adults	4.3 (females), 5.1 (males)	0.4

Children of all prepubertal ages have higher urinary uric acid clearance as well, with a fractional excretion rate of 38–61%, compared with the adult rate of approximately 10%.

These factors make children more susceptible to developing hyperuricosuria (excessive amounts of uric acid in the urine), which may make them more likely to develop urinary uric acid precipitates. The neonatal kidney is, however, less efficient at concentrating and acidifying urine during the first weeks of life, which may help protect the kidney from potentially harmful effects of excreting the relatively large quantities of uric acid. However, increased uric acid load from surgery, drugs or asphyxia could overwhelm the neonatal kidney and pose a potential risk for developing acute urate nephropathy (Stapleton, 1983).

Elevated uric acid levels can also result in nephrolith formation. In fact, uric acid stones are the most common radiolucent kidney stone of children (Fathallah-Shaykh & Neiberger, 2008). Uric acid stone formation has also been associated with hypertension and low urinary pH (Negri et al., 2007; Losito et al., 2009). Factors that lower urinary pH, such as catabolism and acidosis, may promote stone formation as well.

In the case of the 2008 incident, milk was diluted and therefore contained less protein. Not only were infants who drank adulterated formula exposed to high levels of melamine, but they may also have been receiving inadequate protein in their diet. This, along with the fact that infant basal serum uric acid levels and urine filtered levels are higher than those of adults, would increase the likelihood of uric acid stone formation.

Infants, and neonates of most species, have much higher growth rates and consume a larger percentage of food per unit of body weight than do older individuals (Mifflin et al., 1990). In addition, neonates feed much more frequently, sometimes

every 2 h. Considering the half-lives of melamine and other triazines, it is conceivable that the frequency of feeding could increase plasma peak levels due to a repeated dosing effect.

7.2.6 Comments on comparative uric acid metabolism

Humans and most primates do not have the enzyme urate oxidase (uricase), which in most other mammals is responsible for the conversion of uric acid to allantoin (Wu et al., 1989; Watanabe et al., 2002). Consequently, humans have serum uric acid levels that are 10–20 times higher than those in other mammals. Dogs, cats, rats and primates have serum uric acid concentrations in the range 0.1–1.18 mg/dl, compared with adult human values of 4.3–5.1 mg/dl (Fanelli & Beyer, 1974; Teare, 1999). It is therefore important to consider the normal endogenous uric acid concentrations when using animal models for risk assessment. Melamine dosages needed to produce melamine–uric acid stones in normal rats with normal uric acid oxidase function may be higher than what is needed in primates. Further, rats with impaired uric acid oxidase are more susceptible to crystal formation in uric acid nephropathy (Johnson, Stavric & Chartrand, 1969; Stavric, Johnson & Grice, 1969) and also to uric acid stone formation (Lauterburg et al., 1977; Schramek, Heidenreich & Engelmann, 1993). Thus, the lack of uric acid oxidase may be one added risk factor for the human infant.

7.3   Toxicological data

It should be noted that the preliminary results of recent studies show that melamine crystals dissolve rapidly in formalin (R. Reimschuessel et al., unpublished data, 2008). Since all studies prior to 2007 used formalin to preserve tissues for histopathology, crystals may not have been observed in those tissues.

7.3.1 Melamine

7.3.1.1   Acute toxicity

Melamine has low acute toxicity. In male and female rats orally dosed by gavage, a median lethal dose (LD_{50}) of 3161 mg/kg body weight was reported in males, which is the lowest oral LD_{50} in tested rodents (NTP, 1983). The oral LD_{50} for mice was in the same range as that for rats.

7.3.1.2   Short-term studies of toxicity
(a)   Rodents

Male and female rats in a 14-day study were fed melamine at concentrations of 5000, 10 000, 15 000, 20 000 and 30 000 mg/kg[1] in the diet. All animals survived to the end of study. Both male and female rats receiving the middle and high doses (15 000 mg/kg diet and higher) showed reduced body weight and body weight gain, and hard crystalline solid was observed in the urinary bladder in males and females at high doses (20 000 and 30 000 mg/kg diet). Pale and pitted kidneys were also reported in some highest-dose males. The same doses were given to male and female B6C3F1

---

[1] Annex 5 contains a table of conversion factors from mg/kg diet to mg/kg body weight for various species, taken from IPCS (1987).

mice, and hard crystalline materials were found in the urinary bladder of all treated males and some females at the highest dose (NTP, 1983).

The subchronic toxicity of melamine was evaluated in three United States National Toxicology Program (NTP) 13-week oral studies in Fischer 344 rats. In Study I, males and females were fed melamine at dietary concentrations of 0, 6000, 9000, 12 000, 15 000 and 18 000 mg/kg (for details, refer to Table 10 in section 7.4 below). Toxicity included reduced body weight gain and body weight in males receiving 6000 mg/kg diet and in both sexes receiving 12 000 mg/kg diet or more. One male rat receiving 18 000 mg/kg diet and two males receiving 6000 mg/kg diet died. Stones were found in the urinary bladders of treated males in all dosage groups, even in the lowest dose group. The incidence of stone formation was dose related. Epithelial hyperplasia of the urinary bladder was observed in 8/10 males and 2/10 females receiving melamine at 18 000 mg/kg diet, whereas focal epithelial hyperplasia was observed in only 1/10 males and in none of the females receiving 6000 mg/kg diet. Again, as with stone formation, the incidence of hyperplasia appeared to be dose related.

Study II was performed at lower doses to provide dose estimates for the 2-year carcinogenicity study. Males and females were fed melamine at dietary concentrations of 0, 750, 1500, 3000, 6000 and 12 000 mg/kg in the diet. Males at 6000 and 12 000 mg/kg diet showed 10% decreases in weight gain compared with control males. All female dosed groups had the same weight gain as the control females. Feed consumption was not affected in any male or female group. Hyperplasia of the transitional epithelium of the bladder was present in 1/10 male rats at 3000 mg/kg diet, 3/10 male rats at 6000 mg/kg diet and 9/9 male rats at 12 000 mg/kg diet. These changes were found only in the male rats with bladder stones. All dosed groups, including the 750 mg/kg diet group, manifested bladder stones. Dose-related calcareous deposits were observed in the straight segments of the proximal tubules in female rats (3/10 receiving 750 mg/kg diet, 4/10 receiving 1500 mg/kg diet, 10/10 receiving 3000 mg/kg diet, 8/10 receiving 6000 mg/kg diet and 10/10 receiving 12 000 mg/kg diet) (NTP, 1983).

Study III, a 13-week study, was conducted to evaluate the addition of 1% ammonium chloride in control rats and rats given 18 000 mg melamine/kg diet. Stone formation was unaffected by treatment with ammonium chloride (NTP, 1983).

In a mouse study, male and female mice were fed melamine at dietary concentrations ranging from 6000 to 18 000 mg/kg. Body weight gain was reduced in all treated groups. The incidence of bladder stones was dose related in mice as well, and the incidence was greater in males than in females. Ulceration of bladder epithelium was observed and appeared dose related. Sixty per cent of animals with bladder ulcers also exhibited kidney stones. Epithelial hyperplasia was observed only in male mice treated at the highest dose (NTP, 1983).

In a study examining formation of stones by melamine (0.2, 0.4, 0.7, 1.0, 1.3, 1.6 and 1.9% in diet, equal to 2000, 4000, 7000, 10 000, 13 000, 16 000 and 19 000 mg/kg in diet) in weanling rats, Heck & Tyl (1985) found that there was a definite dose–response relationship (see Figure 6). Bladder calculi (stones) were detected at dietary concentrations ranging from 0.4% to 1.9% following an exposure period of 28–29 days. The calculi were composed primarily of melamine and protein, with traces of phosphate, oxalate and uric acid. Histopathology revealed hyperplasia in 94 bladders at dietary doses equal to or greater than 0.7%, 93 of which contained calculi. The authors noted that the incidence of calculi determined by histopathology was much lower than that determined by gross examination. They stated that "these results indicated that conventional methods used to prepare bladder tissue for microscopic

examination often result in the loss of stones" and that this could account for the observation that "lesions have been detected in bladders that did not appear to contain calculi".
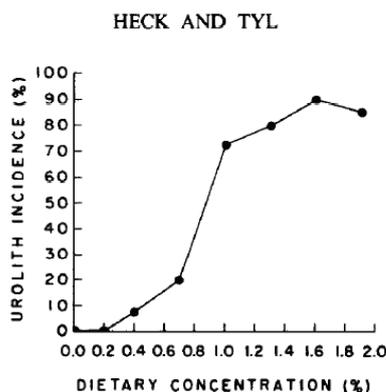
Figure 6. Incidence of urolithiasis in weanling male F344 rats ingesting dietary melamine for 28–29 days (Heck & Tyl, 1985) *[Reprinted from Regulatory Toxicology and Pharmacology, Volume 5, Heck, H.D.'A. & Tyl, R.W. The induction of bladder stones by terephthalic acid, dimethyl terephthalate, and melamine (2,4,6-triamino-s-triazine) and its relevance to risk assessment, Pages 294–313, Copyright (1985), with permission from Elsevier.]*

(b) Other species

There is only one dog study publicly available, and the information is very brief. Dogs were fed with melamine at 1200 mg/kg body weight per day for 1 year. Crystalluria persisted throughout the entire study. One of these dogs developed a large bladder stone and had evidence of chronic cystitis (IUCLID, 2000; Bingham, Cohrssen & Powell, 2001).

A study with sheep receiving melamine reported melamine-induced crystalluria and mortality (Clark, 1966). MacKenzie (1966) also reported weight loss and mortalities in sheep fed melamine, although the cause of death was not determined. Serious limitations of the experimental designs in each of these studies precluded the use of the data in risk assessment of melamine in ruminants.

7.3.1.3   Long-term studies of toxicity and carcinogenicity

In an NTP carcinogenicity study, rats were fed with melamine at 0, 4500 or 9000 mg/kg in the diet for females and 0, 2250 or 4500 mg/kg in the diet for males for 2 years. At the end of the study, there was no significant difference in survival between all groups. Transitional cell carcinomas in the urinary bladder of treated males showed a statistically significant positive trend. Chronic inflammation, dose-related interstitial lymphoplasmacytic infiltration and cortical fibrosis in the kidney were observed in treated females. C-cell carcinoma of the thyroid was observed in treated females with a statistically significant positive trend. On the other hand, pancreatic islet cell carcinomas in males and endometrial stromal polyps in females were observed with a statistically significant negative trend (NTP, 1983).

In a concurrent mouse study, mice were fed melamine at 0, 2250 or 4500 mg/kg in the diet. A lowered survival rate was observed in the high-dose males. Stones (calculi) and acute/chronic inflammatory and hyperplastic changes in urinary bladder were found in treated males. Those changes were also found in the high-dose females but with a much lower incidence rate. No neoplasia was detected in the female bladders (NTP, 1983).

Male rats were fed with 3% or 1% thymine or 3%, 1% or 0.3% melamine (purity, >99%; equal to 30 000, 10 000 or 3000 mg/kg) in the diet for 36 weeks followed by a 4-week recovery period. In addition to calculi, both carcinomas and papillomas were detected in urinary bladders and ureters. The results showed that melamine induces calculus formation and that the incidence is dose related: 100% in the 3% group and 70% in the 0.3% group. It also showed that the incidences of carcinomas and papillomas of bladder and ureter are melamine treatment and dose related (Okumura et al., 1992).

The effects of sodium chloride on melamine-induced calculi and proliferation of lesions in the kidney were evaluated in rats. Animals were fed with 3% or 1% melamine (equal to 30 000 or 10 000 mg/kg) alone in the diet, combined with either 10% or 5% sodium chloride (100 000 or 50 000 mg/kg in the diet) or fed with 10% sodium chloride alone for 36 weeks followed by a 4-week recovery period. Clinical signs noted in the 3% dosed animals included decreased food consumption with slight weight loss and an increase in urine volume and decreased osmolality. At the end of the study, calculi were observed, and analysis indicated that the composition was melamine and uric acid in an equimolar ratio. The results showed a suppressive effect of sodium chloride on melamine-induced calculus formation and hyperplasia of the papilla in the kidney. Microcrystals were observed in urine sediments. Histopathology noted ischaemic changes with focal fibrosis, inflammation and renal tubule regeneration (Ogasawara et al., 1995).

In one study not examining renal toxicity, Hendry, Rose & Walpole (1951) noted that melamine (1750 mg/kg by mouth in suspension for 14 days) appeared to promote growth of implanted Walker tumours in albino rats. The neoplasms were 20% heavier in melamine-treated rats than those of the untreated controls.

The International Agency for Research on Cancer (IARC, 1999) concluded that the non-deoxyribonucleic acid (DNA)-reactive mechanism by which melamine produced urinary bladder tumours in male rats occurred only under conditions in which calculi were produced. Overall, melamine was not classifiable as to its carcinogenicity to humans. After chronic exposure, melamine has induced proliferative epithelial lesions, mainly located at the proximal end of the urinary tract, sometimes in the absence of observable calculi (Melnick et al., 1984; Cremonezzi et al., 2004). However, as indicated, this may be due to loss of calculi as a result of the tissue preparation method.

7.3.1.4 Genotoxicity

Several abstracts report that melamine was not mutagenic in a bacterial mutagenicity test (*Salmonella typhimurium*), in sister chromatid exchange in Chinese hamster ovary cells in vitro or in an in vivo micronucleus assay (Mast, Naismith & Friedman, 1982; Mast et al., 1983; IARC, 1999).

7.3.1.5 Reproductive and developmental toxicity

There was no evidence of adverse effects on reproductive organs in a study by Melnick et al. (1984), in the 13-week toxicity studies or in carcinogenicity studies described above (NTP, 1983). Melamine is not teratogenic in the rat. The no-observed-adverse-effect level (NOAEL) is about 1060 mg/kg body weight per day for fetal toxicity and about 400 mg/kg body weight per day for maternal toxicity (Helwig, Gembrandt & Hildebrandt, 1996). The fetal toxicity may have been mediated by the maternal toxicity.

7.3.2 Cyanuric acid

7.3.2.1 Acute toxicity

Cyanuric acid has low acute oral toxicity. The lowest oral LD_{50}s reported were 7700 mg/kg body weight in rats and 3400 mg/kg body weight in mice (OECD, 1999).

7.3.2.2 Short-term studies of toxicity

In 13-week studies in mice given sodium cyanurate at concentrations up to 5375 mg/l (equivalent to 1500 mg/kg body weight per day) in drinking-water, the only compound-related effect reported was the occurrence of bladder calculi in males receiving the highest dose. In a similar study in rats, 1/28 males in the group receiving 1792 mg/l (equivalent to 145 mg/kg body weight per day) and 7/28 males in the group receiving the highest dose (equivalent to 495 mg/kg body weight per day) showed epithelial hyperplasia of the bladder (WHO, 2004).

In an early study, male and female rats were orally treated with sodium cyanurate at 0.8% or 8% in diet (equal to 8000 and 80 000 mg/kg) for 20 weeks. Mortality was observed in both high and low dose groups—70% and 20%, respectively. No changes were observed in haematological examination or organ weights (except kidney). Histological study revealed dilated distal collecting tubules and ducts of Bellini with focal areas of epithelial proliferation in the high dose group (OECD, 1999).

In a 6-month study of white rats and guinea-pigs given cyanuric acid at 0.3, 3 or 30 mg/kg body weight orally (Mazaev, 1962), histology showed "some dystrophic changes in the parenchyma of the kidneys" in all the test animals that had received cyanuric acid at the dose of 30 mg/kg body weight and "dystrophy of the heart muscle" in some of the animals. No changes were noted in animals receiving 0.3 or 3 mg/kg body weight.

Several early studies were performed in ruminants; however, owing to limitations in the studies and their reporting, these are not further considered.

7.3.2.3 Long-term studies of toxicity and carcinogenicity

In a 2-year study, rats were given sodium cyanurate in the drinking-water at doses estimated as 0, 26, 77, 154 or 371 mg/kg body weight per day (0, 400, 1200, 2400 and 5375 mg/l), with control groups receiving drinking-water containing an equivalent amount of sodium hippurate or untreated drinking-water. Survival was slightly lower in the group receiving the highest dose compared with the control group receiving untreated drinking-water, but not the control group receiving sodium hippurate. This was attributed to the development of calculi in the urinary tract. Male rats were anatomically more susceptible to blockage from calculi. There was no substance-related increase in tumour incidence. Multiple lesions of the urinary tract secondary to urinary tract irritation and obstruction (calculi and hyperplasia, bleeding and inflammation of the bladder epithelium, dilated and inflamed ureters and renal tubular nephrosis) and cardiac lesions (acute myocarditis, necrosis and vascular mineralization) were reported in males that died during the first year of the study and that were receiving a dose of 371 mg/kg body weight per day. In females, no bladder calculi were observed in the first year. Inflammatory lesions of the heart were apparent in some high-dose males that died earlier. No toxicologically significant treatment-related effects were observed at 154 mg/kg body weight, which was considered to be the NOAEL in this study (WHO, 2004).

In a similar 2-year study in which mice received doses of sodium cyanurate equivalent to 0, 30, 110, 340 or 1523 mg/kg body weight per day (0, 400, 1200, 2400

and 5375 mg/l), survival was similar in all groups, and there were no treatment-related changes in the incidence of tumours or other histopathological lesions (WHO, 2004).

Limited information is available for dogs treated with cyanuric acid. In a 2-year chronic toxicity study, several dogs fed at 8% (equal to 80 000 mg/kg in diet) died. Gross and microscopic pathologies were noted in the kidneys (Hodge et al., 1965).

7.3.2.4 Developmental and reproductive toxicity

No evidence of treatment-related fetotoxicity or maternal toxicity was apparent in rabbits dosed by gavage with sodium cyanurate daily at 0, 50, 200 or 500 mg/kg body weight per day from gestation days 6 to 18 and in rats dosed by gavage with sodium cyanurate daily at 0, 200, 1000 or 5000 mg/kg body weight per day from gestation days 6 to 15 (Cascieri et al., 1985; Hammond et al., 1986).

No treatment-related adverse effects were observed in rats treated with 0, 400, 1200 or 5375 mg/l (maximum solubility) of sodium cyanurate in drinking-water from 36 days of age for parents and continued for at least 100 days before mating. A few high-dose males exhibited calculi in the urinary bladder, with microscopic evidence of epithelial hyperplasia or chronic cystitis (Wheeler et al., 1985; Hammond et al., 1986). A further study in rats dosed by gavage with sodium cyanurate at 0, 10, 40, 150 or 600 mg/kg body weight per day from 14 days before mating up to day 3 of lactation for females, for a total of 44 days, also did not show any maternal or offspring toxicity (OECD, 1999).

7.3.2.5 Genotoxicity

Cyanuric acid was considered non-genotoxic in an adequate battery of in vitro and in vivo tests (WHO, 2004).

7.3.3 *Melamine plus cyanuric acid and other structural analogues*

The effects of exposure to a combination of triazines, especially melamine and cyanuric acid, were apparent in the many cat and dog cases reported in 2007; those animals developed renal crystals, which caused kidney failure (Brown et al., 2007; Reyers, 2007; Cianciolo et al., 2008; Thompson et al., 2008). Thousands of pigs died after being fed diets containing melamine at 3026 mg/kg, ammeline at 958 mg/kg and cyanuric acid at 69 031 mg/kg (Luengyosluechakul, 2007).

Oral exposure to melamine given simultaneously with cyanuric acid caused much more severe renal damage than did oral exposure to melamine or cyanuric acid alone. Rats treated orally with melamine at 50 mg/kg body weight per day in combination with the same dose of cyanuric acid for 3 days presented increased blood urea nitrogen and creatinine in blood with increased kidney weight and crystals in kidney, whereas there were no changes in animals treated with only melamine at 50 mg/kg body weight per day or with a combination of melamine and cyanuric acid at 5 mg/kg body weight per day each (Kim et al., 2008). Rats given a mixture of four triazines (melamine at 400 mg/kg body weight per day and ammeline, ammelide and cyanuric acid, each at 40 mg/kg body weight per day) or the combination of melamine and cyanuric acid (each at 400 mg/kg body weight per day) for 3 days had elevated serum blood urea nitrogen and creatinine and increased kidney weights (Dobson et al., 2008). Rats given a single dose of either ammeline or ammelide (0, 10, 30 or 100 mg/kg body weight) alone and evaluated 24 h after dosing had no changes in kidney weight, blood urea nitrogen, serum creatinine or creatinine clearance (Dobson et al., 2008).

Cats given food containing 32, 121 or 180 mg/kg body weight of both melamine and cyanuric acid for 2 days showed acute renal failure (elevated blood urea nitrogen and creatinine), with crystals in urine and kidneys. There were, however, no changes observed in cats treated with similar doses of melamine or cyanuric acid singly for longer time periods (Puschner et al., 2007).

Pigs given both melamine and cyanuric acid (400 mg/kg body weight each) orally had elevated serum blood urea nitrogen and creatinine and developed renal crystals. No clinical changes or crystals were observed in pigs treated with the same dose of melamine or cyanuric acid separately (S. Ensley, personal communication, 2008; Reimschuessel et al., 2008).

Fish given an oral dose of a mixture of melamine and cyanuric acid (400 mg/kg body weight each) for 3 days developed extensive crystals in their renal tubules, but animals treated with the same dose of melamine or cyanuric acid separately did not develop crystals. Crystals have also been reported to form in trout given melamine first (20 mg/kg body weight per day for 3 days), followed by a single dose of cyanuric acid (20 mg/kg body weight) 6 days later (Reimschuessel et al., 2008). The excretion of many chemicals is slower in fish than in mammals. The relevance of these data to mammals is unknown.

An acute threshold dose for the combination of melamine and cyanuric acid administered together in rats is 5 mg/kg body weight per day for 3 days (Kim et al., 2008). Preliminary data on a limited number of fish indicate that in catfish, a 4-day threshold dose is 2.5 mg/kg body weight per day; however, if this dose is given for 14 days, the fish develop crystals (R. Reimschuessel et al., unpublished data, 2008).

Sheep given ammeline or ammelide with various concentrations of melamine developed crystalluria and renal failure and died (MacKenzie & van Rensburg, 1968).

There were no data on the long-term toxicity, carcinogenicity or genotoxicity of co-administered melamine and cyanuric acid or analogues.

Data on the toxicity of the melamine–cyanurate complex are limited. One study describing the acute oral toxicity of melamine–cyanurate in rats and mice found the LD_{50}s to be 4110 mg/kg body weight and 3461 mg/kg body weight, respectively (Babayan & Aleksandryan, 1985), compared with the toxicity of the individual compounds (melamine: 6000 mg/kg body weight in rats, 4282 mg/kg body weight in mice; cyanuric acid: 7667 mg/kg body weight in rats, 3378 mg/kg body weight in mice). This would indicate that the melamine–cyanurate complex is somewhat more toxic than each chemical alone; however, it is still much less toxic than when the two chemicals are co-administered as individual compounds. In that case, as was seen in experimental studies, doses as low as 32 mg/kg body weight caused toxicity in cats (Puschner et al., 2007).

7.4   Mechanism of crystal-induced renal failure

Renal failure caused by the formation of intrarenal uric acid crystals has been described in humans and animals and termed "acute uric acid nephropathy" (Casdorph, 1964; Conger, 1990). The precipitation of crystals in the kidney occurs in association with an acidic urinary pH of less than 5.5, an increasing concentration of uric acid and dehydration (Conger & Falk, 1977; Davidson et al., 2004; Tiu et al., 2007). Davidson et al. (2004) stated that with certain diseases "the threshold at which uric acid precipitates into crystals may be reached. These crystals obstruct urine flow in the tubules, leading to uric acid nephropathy." The authors, however, did not give a threshold level at which to expect this precipitation. Cases of lethal uric acid nephropathy have reported serum uric acid values of 80 and 185 mg/dl (Casdorph,

1964); however, uric acid levels of 12–20 mg/dl have also been reported in cases of acute uric acid nephropathy in children following cardiovascular surgery during which uric acid levels rise (Ejaz et al., 2007). Patients develop oliguria or anuria with elevated serum blood urea nitrogen and creatinine.

Renal failure results from both intrarenal crystal-associated obstruction and an elevation in renal pressure that reduces renal blood flow and glomerular filtration. Extrarenal uric acid precipitation may also occur in the ureter or renal pelvis, further obstructing urinary flow (Casdorph, 1964; Conger et al., 1976). Chronic changes found in human kidneys include local granulomatous inflammation associated with macrophage and T-cell infiltration (Ejaz et al., 2007). Similar changes are found in experimental rat and mouse models, including intratubular urate crystals and inflammatory infiltrates with associated necrotic debris (Stavric, Johnson & Grice, 1969; Wu et al., 1994).

The crystal-induced nephropathy seen in animals exposed to melamine and cyanuric acid appears to be similar to uric acid nephropathy, in that it is a mechanical obstruction that results in renal damage, rather than a systemic toxic effect. Crystals composed of melamine–cyanurate precipitate in renal tubules following ingestion of both of these triazines simultaneously by cats, rats, pigs and fish (Puschner et al., 2007; Dobson et al., 2008; Kim et al., 2008; Reimschuessel et al., 2008). The characteristic layered, spherical crystal conglomerates with a radial crystal pattern are seen obstructing tubules in wet mount tissue sections. The appearance of these crystals is similar to that of uric acid crystal "spherulites" or "spherically symmetrical, radiating crystal aggregates" that can occur in humans with gout (Fiechtner & Simkin, 1980, 1981) or that occur in the renal sac of the ascidian *Corella inflata* (Lambert et al., 1998).

Chronic cases show interstitial fibrosis and inflammation associated with melamine–cyanurate renal crystals (Brown et al., 2007; Cianciolo et al., 2008; Reimschuessel et al., 2008; Thompson et al., 2008). These lesions are similar to chronic lesions associated with uric acid crystal nephropathy in humans or animals and are characterized by typical renal responses to obstruction and foreign body reactions.

Identifying crystal nephropathy that is not experimentally induced poses a diagnostic dilemma. It is likely that individual crystals, crystal aggregates and smaller stones/calculi would not be identified or found by ultrasound, owing to the limits of resolution of the method. These are microscopic changes. Only gross structural changes in the kidney, changes in overall medullary density (Kenney, 1991) or stones larger than 1 mm would be seen with ultrasound. Additional methods to visualize early changes in kidneys, including diffuse calcareous deposits such as those seen in female rat kidneys in the NTP (1983) study, are needed.

7.5   Dose–response considerations

The lowest oral dose identified for general toxic effects was ≤63 mg/kg body weight per day, based on the toxicological significance of minimal urolithiasis (urinary tract stone/crystal formation) induction in Fischer 344 male rats following dietary melamine exposure for 13 weeks (combined dose range from two studies was 750–18 000 mg/kg) (NTP, 1983; Melnick et al., 1984). Combined analysis of data from the two separate studies showed consistent responses for both bladder stone

formation and the reported incidence of bladder epithelium hyperplasia (Table 10). Similar effects were observed in female rats, but at a lower incidence rate.

Table 10. Survival and bladder lesions in male F344 rats fed diets containing melamine in three separate 13-week studies (NTP, 1983)

Dose (mg/kg diet)	Survival	Incidence of urinary bladder stones[a]	Incidence of hyperplasia of the bladder epithelium[a]
Study I			
0	12/12	0/12	1/10
6000	10/12	6/12	4/7
9000	12/12	8/12	NE
12 000	12/12	12/12	NE
15 000	12/12	10/12	NE
18 000	11/12	12/12	10/10
Study II			
0	10/10	1/10	1/8
750	10/10	2/10	4/9
1500	10/10	5/10	1/10
3000	10/10	7/10	6/10
6000	10/10	9/10	6/10
12 000	10/10	9/9	8/9
Study III			
0	10/10	1/10	NE
0 + ammonium chloride 1%	10/10	0/10	NE
18 000	10/10	10/10	NE
18 000 + ammonium chloride 1%	8/10	8/8	NE

NE, not evaluated.

[a] Number of animals with lesion/number of animals examined. Slides of the urinary bladder of male rats were re-examined in a blind (coded) fashion after completion of the 2-year dosed feed study of melamine, as the urinary bladder was the target organ in that study. The data presented in the last column of this table differ slightly from the data of the NTP technical report on the carcinogenesis bioassay of melamine (NTP, 1983), in that the incidences of hyperplasia of the bladder epithelium were greater for most of the treatment groups based on re-examination of the tissues (Melnick et al., 1984).

While there was a high incidence of calcareous deposits noted in the kidneys of female rats following subchronic exposure to melamine (NTP, 1983), this was not a consistent finding (observed in only one study).

In comparison, chronic exposure of rats to melamine (2250 or 4500 mg/kg diet) produced lower rates of bladder stone formation in both sexes, but a dose-dependent increase in chronic kidney inflammation and nephropathy (fibrosis) in female rats (NTP, 1983).

Compared with the chronic studies (only two doses), multiple dose groups were used in the subchronic studies, with an extensive dose range (24-fold). As melamine does not bioaccumulate, has a short half-life and does not induce systemic

effects, the Expert Meeting decided that studies with a shorter duration of exposure could be used for chronic risk assessment purposes. The lowest dose in rats from the subchronic studies was 750 mg/kg in the diet and resulted in 2/10 male rats with bladder stones compared with the combined controls, which resulted in 1/22 (non-significant, $P > 0.05$, Fisher's exact test). However, the dose–response trend in these data is clearly significant ($P < 0.01$), and the response in male rats at 1500 mg/kg diet (the second lowest dose used) is significant when compared with the combined controls ($P < 0.01$). Further support for selection of bladder stone formation as the critical end-point was obtained from the study of Heck & Tyl (1985), which provided evidence for a threshold or NOAEL (0.2% in the diet, or 2000 mg/kg diet) following a shorter exposure period (28–29 days).

As an alternative to the traditional NOAEL approach, dose–response modelling was selected in order to fully capture the general pattern of the dose–response. Dose–response analysis was conducted using the Benchmark Dose Software from the USEPA (version 2.0). For all of the available data, three separate model types were employed: quantal linear, multistage and the Weibull model. These three models range from a rather conservative model (linear) to a model that allows for a threshold type of dose–response (Weibull), whereas the other model (quadratic) falls in between. In some cases, other models (e.g. log-logistic, probit) could yield more conservative or more threshold-like patterns than these three models; however, as there is some underlying biological support for the three models chosen, the evaluation was based on these three. These models are described in more detail on the USEPA Benchmark Dose Software web site (http://www.epa.gov/NCEA/bmds/index.html).

Two response levels were used in calculating benchmark doses (BMD) and their 95% lower bounds (BMDL): 10% (BMD_{10}, $BMDL_{10}$) and 5% (BMD_{05}, $BMDL_{05}$). The patterns seen in the benchmark doses appear to be similar for the BMD_{05} and the BMD_{10}. 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 NTP (1983) studies, it was suggested that a $BMDL_{10}$ for bladder stone development would be an appropriate toxicological reference point. In addition, a $BMDL_{10}$ has been suggested as appropriate for substituting for the NOAEL when calculating reference doses such as the acceptable daily intake (ADI) or TDI (Barnes et al., 1995).

All three models resulted in a BMD_{10} of 531 mg/kg diet and a $BMDL_{10}$ of 415 mg/kg diet.[1] Dietary conversion used the standard mg/kg diet to mg/kg body weight per day conversion factors (see Annex 5) but also considered an additional feed intake reduction factor of 14% as observed in the second subchronic study (NTP, 1983). This results in doses of approximately 44.6 mg/kg body weight per day and 35 mg/kg body weight per day for the 10% risk of bladder stone formation (BMD_{10} and $BMDL_{10}$, respectively). No renal histopathological effects were observed in the

---

[1] Note that in our analysis, we restricted the power of the Weibull model to be >1 as suggested by Crump (1984) in his original paper on benchmark dose estimation; without this restriction, the Weibull model becomes supralinear (it has an infinite slope at dose 0) and biologically implausible. This supralinear behaviour yields a smaller BMD and BMDL, which we have chosen not to consider because of the biological implausibility of an infinite slope at dose 0. The log-logistic model also results in a supralinear model form and yields lower BMDs and BMDLs than the values used here, which we have not included because of their biological implausibility.

subchronic studies, and urinalysis of the animals in the 750 mg/kg diet group revealed no significant differences from controls or the presence of melamine crystalluria.

For dose comparison, no bladder stones were observed in weanling rats (28 days old) exposed to melamine at a dietary concentration of 0.2% (2000 mg/kg) for 4 weeks in the supporting study by Heck & Tyl (1985) noted above. This is equivalent to a dose of approximately 200 mg/kg body weight per day, assuming a standard dietary conversion factor of 10 (Annex 5). A $BMDL_{10}$ estimated from this study for bladder stone development is approximately 384 mg/kg body weight per day (0.384% diet), which is more than 10 times higher than the dose of 35 mg/kg body weight per day estimated to result in an incidence of 10% bladder stone formation in rats for subchronic exposure. This suggests that the $BMDL_{10}$ value of 35 mg/kg body weight per day is a conservative value.

8. RISK ASSESSMENT

8.1 Derivation of the tolerable daily intake (TDI)

The $BMDL_{10}$ of 35 mg/kg body weight per day was selected as the toxicological point of departure on which to develop a TDI.

As a first step, the default uncertainty factor of 100 for defining intra- and interspecies variability was selected (IPCS, 1987). This was judged to be adequate in describing the anticipated toxicodynamic and toxicokinetic differences both between rats and humans and within the human population. In particular, differences in serum and urinary uric acid levels, a possible risk factor for melamine–cyanurate crystal formation, between adults and infants are within a factor of 10 (see Table 9 in section 7.2.5). Differences in purine metabolism (lack of uric acid oxidase) between primates, including humans, and rodents, which result in higher circulating serum levels of uric acid in humans, are within a factor of 10–20.

The Expert Meeting noted that the toxicological point of departure, the $BMDL_{10}$ of 35 mg/kg body weight, is likely to be conservative. However, in order to fully account for the potential increased sensitivity of infants and for data uncertainties, an additional 2-fold factor was applied to the default 100-fold uncertainty factor. The uncertainty in data stems from studies demonstrating that formalin preparation of tissues may lead to significant loss of crystals or stones, which could then result in underestimation of stone formation and thus the toxicity of the dose tested.

The TDI was calculated as follows:

$$TDI \quad = \quad \frac{BMDL_{10}}{\text{uncertainty factor}}$$

$$= \quad \frac{35 \text{ mg/kg body weight per day}}{200}$$

$$= \quad 0.175 \text{ mg/kg body weight per day}$$

$$= \quad 0.2 \text{ mg/kg body weight per day (rounded value)}$$

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 previously been derived by WHO (2007), suggesting that exposure to these analogues separately would be no more toxic than exposure to melamine. Available data indicate that simultaneous exposure to melamine and cyanuric acid is more toxic than exposures to each compound individually. Data are not adequate to allow the calculation of a health-based guidance value for this co-exposure.

The TDI for melamine is applicable to infants, as the specific sensitivity of this sensitive population group has been considered, as described above.

8.2 Risk characterization

Melamine and structurally related triazines, such as cyanuric acid, induce a low to moderate degree of toxicity in most experimental animals. Melamine tends to be readily absorbed from the gastrointestinal tract and excreted unmetabolized through the urine. Melamine does not appear to have bioaccumulation potential in mammalian species, and plasma half-lives in rats have been measured in hours (approximately 3 h). Systemic effects are usually not observed; organ-specific toxicity is related to the excretory route, mainly the kidneys and bladder.

The main toxic effects in experimental animals tend to be urolithiasis or bladder stone development, with some associated evidence for kidney toxicity. The incidence of bladder stones was dose related, which would imply that actual stone development requires a critical level of the administered test chemical to be present prior to stone formation. Bladder stones from animals exposed to melamine in the diet were reported to be composed mainly of melamine and protein, with traces of phosphate, oxalate and uric acid (Heck & Tyl, 1985). Although chronic exposure to melamine does induce malignancies in the urinary bladder, this has been postulated to be secondary to the reactive hyperplasia that develops in response to a localized tissue irritation effect, which then progresses to bladder neoplasia. A thresholded mechanism for cancer development by melamine is further supported by the lack of any mutagenic or genotoxic activity in standard assays (point mutation, chromosome aberration, DNA damage, cell transformation). Renal lesions have been reported in some studies, including epithelial proliferative changes and renal papillary mineralization. It is unknown if such mineralization may slough and provide a nidus for stones to form in the bladder.

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

In human infants who consumed adulterated infant formula, the main toxic effect observed was the development of renal calculi, which were composed of a combination of uric acid and melamine (1.2:1 to 2.1:1 range, $n = 15$). Human infants generally have approximately 5-fold higher levels of uric acid compared with rat species (350 μmol/l versus 50–70 μmol/l; 5.88 mg/dl versus 0.84–1.18 mg/dl). This would imply that human infants may be specifically more sensitive than rats to uric acid–melamine kidney stone development.

The dietary exposure of infants 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 renal toxicity observed 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 human adults (weighing 60 kg) who are exposed to contaminated food products, a level of dietary contamination with melamine of all solid food (1.5 kg) at 8 mg/kg would result in consumption at the TDI of 0.2 mg/kg body weight per day.

This conservative estimate of dose would apply to virtually any population class, as it assumes melamine contamination of all solid food, regardless of nature or type.

Although a TDI was not developed for the related triazine compounds ammelide and ammeline, it is anticipated, based on the existing data set, that each alone would be no more toxic than melamine. In addition, this assessment is specific to exposure to melamine alone. Data from experimental animals and pets have shown that co-exposure, in particular to melamine and cyanuric acid, results in renal crystal formation and subsequent kidney damage. Currently, there are insufficient data on which to develop a hazard characterization for these combined exposure scenarios, but it is anticipated that they would be more toxic than separate exposures.

8.3   Considerations for risk management

Development of a TDI does not imply 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. Modestly exceeding the TDI on an occasional basis is not likely to be of health concern. Prolonged exposures above the TDI may be of health concern. The amount and duration of exposures above the TDI that are likely to be without effect are compound specific and depend on the circumstances of exposure.

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.

9.1 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 π-π aromatic ring stacking interactions with cyanuric acid and other analogues, as well as with 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. They were also 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 USA, 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 between melamine and uric acid in vivo and in vitro.

9.2 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, at the time of writing, 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.

LC-MS/MS and GC-MS/MS are the techniques of choice for confirmatory analysis of melamine and its analogues because of their 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.
- In order to avoid adulteration with cheap non-protein nitrogen sources, development of more specific, rapid and low-cost methods for protein analysis that do not include non-protein nitrogen is needed.

9.3   Occurrence of melamine and its analogues in food and feed

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 on baseline levels of melamine originating from certain sources, such as migration from tableware, were available, data on the occurrence of melamine from other sources, such as the use of the pesticide cyromazine or the use of melamine-containing 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 the adulteration of infant formula in China, government laboratories worldwide analysed food and feed samples with a large focus on products containing milk and/or milk-derived ingredients. 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 the presence of 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 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, ammelide, ammeline) in food, feed and feed ingredients, rather than for melamine only.

Recommendations for future work
Further investigate:
- levels of melamine in food and feed resulting from the use of cyromazine and other triazine compounds as a pesticide or veterinary drug;
- the extent of use of melamine and its analogues as (approved) additives for animal feed and in fertilizer for different countries to determine expected baseline levels;
- the carry-over ratio of melamine and its 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.

9.4   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, as 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 on very conservative assumptions relating to the use of sodium dichloroisocyanurate to disinfect drinking-water.

The dietary exposures 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 that 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.

9.5   Epidemiological and toxicological 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, alkalinization of urine (to reach a 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 appear to have developed stones primarily in the urinary tract, which sometimes led to obstructive renal failure (Sun et al., 2008; Guan et al., 2009). Pets, however, exposed to the combination of melamine and cyanuric acid, formed crystals in renal tubules, developing an intra-tubular obstructive nephropathy. Pets developed acute renal failure within 2 days of exposure in severe exposures, whereas most infants with stones reportedly did not have overt clinical symptoms (Guan et al., 2009).

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 testing recommendations presented below are to 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. Study the influence of pH on the solubility of melamine-induced stones for treatment purposes.[1]
- 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 exposure to low doses of melamine or short-term exposure to 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.

---

[1] The group is aware of studies under way or planned by the USFDA in swine and rats to determine a threshold for combined exposure to cyanuric acid and melamine. Also, studies in cows are planned at the University of California–Davis, in the Republic of Korea and in South Africa.

9.6   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 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 $BMDL_{10}$) would be an appropriate toxicological end-point. The $BMDL_{10}$ 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 200 mg/kg body weight per day for 4 weeks.

This $BMDL_{10}$ can be used to estimate the 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.

Recommendation
- WHO should re-evaluate whether a health-based guidance value for co-exposure to melamine and cyanuric acid can be derived once more dose–response data on the combined exposures become available.

9.7   Risk characterization

The dietary exposures based on the consumption of melamine-adulterated infant formula in China at the median levels of melamine reported in the most contaminated brand were 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 renal toxicity observed 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.

9.8 Risk management

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. Modestly exceeding the TDI on an occasional basis is not likely to be of health concern. Prolonged exposures above the TDI may be of health concern. The amount and duration of exposures above the TDI that are likely to be without effect are compound specific and depend on the circumstances of exposure.

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.

10. REFERENCES

Allen LM, Briggle TV, Pfaffenberger CD (1982). Absorption and excretion of cyanuric acid in long-distance swimmers. *Drug Metabolism Reviews*, 13: 499–516.

Andersen WC et al. (2008). Determination and confirmation of melamine residues in catfish, trout, tilapia, salmon, and shrimp by liquid chromatography with tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*, 56: 4340–4347.

Babayan EA, Aleksandryan AV (1985). Toxicological characteristics of melamine cyanurate, melamine and cyanuric acid. *Zhurnal Eksperimental'noi i Klinicheskoi Meditsiny*, 25(4): 345–349.

Barbee SJ et al. (1984). Metabolism and disposition of sodium cyanurate in the dog. *Toxicologist*, 4: 92.

Barboza D, Barrionuevo A (2007). Filler in animal feed is open secret in China. *The New York Times*, 30 April 2007 (http://www.nytimes.com/2007/04/30/business/worldbusiness/30food.html).

Barnes D et al. (1995). Benchmark dose workshop: Criteria for use of a benchmark dose to estimate a reference dose. *Regulatory Toxicology and Pharmacology*, 21: 291–306.

Baynes RE et al. (2008). Pharmacokinetics of melamine in pigs following intravenous administration. *Food and Chemical Toxicology*, 46(3): 1196–1200.

BERNAMA (2008). *Malaysia bans import of raising agent from China, orders recall of two biscuit brands*. Malaysian National News Agency, 16 October 2008 (http://www.bernama.com/bernama/v3/news_lite.php?id=364989).

Bingham E, Cohrssen B, Powell CH, eds. (2001). *Patty's toxicology*, 5th ed. *Vol. IV. Hydrocarbons/organic nitrogen compounds*. New York, NY, John Wiley & Sons.

Bizzari S, Yokose K (2008). Melamine. In: *Chemical economics handbook*. Menlo Park, CA, SRI Consulting, Inc. (http://www.sriconsulting.com/CEH/Public/Reports/673.3000/; accessed 17 November 2008).

Bradley EL et al. (2005). Survey of the migration of melamine and formaldehyde from melamine food contact articles available on the UK market. *Food Additives and Contaminants*, 22(6): 597–606.

Brady AP, Sancier KM, Sirine G (1963). Equilibria in solutions of cyanuric acid and its chlorinated derivatives. *Journal of the American Chemical Society*, 85: 3101–3104.

Brown CA et al. (2007). Outbreaks of renal failure associated with melamine and cyanuric acid in dogs and cats in 2004 and 2007. *Journal of Veterinary Diagnostic Investigation*, 19: 525–531.

Cascieri T et al. (1985). Comprehensive evaluation of the urinary tract after chronic exposure to cyanurate in drinking water. *Toxicologist*, 5: 58.

Casdorph HR (1964). Acute uric acid nephropathy in leukemia. *California Medicine*, 101: 481–484.

Cattaneo P, Ceriani L (1988). Melamine in animal meals. *Tecnica Molitoria*, 39: 28–32.

Chan EYY, Griffiths SM, Chan CW (2008). Public-health risks of melamine in milk products. *Lancet*, 372: 1444–1445.

Chen S-K, Kang J-J (2008). *Surveillance report on melamine contamination in Taiwan—Analysis of melamine contamination in milk powder*. Taiwan, China, Institute of Toxicology, College of Medicine, National Taiwan University; Bureau of Food and Drug Analysis, Department of Health; Centers of Disease Control, Department of Health; Taipei Hospital, Department of Health. Unpublished report submitted to WHO.

Chou S-S, Hwang D-F, Lee H-F (2003). High performance liquid chromatographic determination of cyromazine and its derivative melamine in poultry meats and eggs. *Journal of Food and Drug Analysis*, 11(4): 290–295.

Cianciolo RE et al. (2008). Clinicopathologic, histologic, and toxicologic findings in 70 cats inadvertently exposed to pet food contaminated with melamine and cyanuric acid. *Journal of the American Veterinary Medical Association*, 233(5): 729–737.

Clark R (1966). Melamine crystalluria in sheep. *Journal of the South African Veterinary Medical Association*, 37: 349–351.

Conger JD (1990). Acute uric acid nephropathy. *Medical Clinics of North America*, 74(4): 859–871.

Conger JD, Falk SA (1977). Intrarenal dynamics in the pathogenesis and prevention of acute urate nephropathy. *Journal of Clinical Investigation*, 59: 786–793.

Conger JD et al. (1976). A micropuncture study of the early phase of acute urate nephropathy. *Journal of Clinical Investigation*, 58: 681–689.

Cremonezzi DC et al. (2001). Dietary polyunsaturated fatty acids (PUFA) differentially modulate melamine-induced preneoplastic urothelial proliferation and apoptosis in mice. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 64(3): 151–159.

Cremonezzi DC et al. (2004). Neoplastic and preneoplastic lesions induced by melamine in rat urothelium are modulated by dietary polyunsaturated fatty acids. *Food and Chemical Toxicology*, 42(12): 1999–2007.

Crump KS (1984). A new method for determining allowable daily intakes. *Fundamental and Applied Toxicology*, 4(5): 854–871.

Davidson MB et al. (2004). Pathophysiology, clinical consequences, and treatment of tumor lysis syndrome. *American Journal of Medicine*, 116: 546–554.

Dobson RL 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*, 106: 251–262.

EFSA (2007). *EFSA's provisional statement on a request from the European Commission related to melamine and structurally related compounds such as cyanuric acid in protein-rich ingredients used for feed and food*. Parma, European Food Safety Authority, 4 July 2007 (Question No. EFSA-Q-2007-093).

EFSA (2008a). *Statement of the EFSA on risks for public health due to the presence of melamine in infant milk and other milk products in China*. Parma, European Food Safety Authority, 24 September 2008 (Question No. EFSA-Q-2008-695). *EFSA Journal*, 807: 1–10 (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902098495.htm).

EFSA (2008b). *Concise European Food Consumption Database*. Parma, European Food Safety Authority (http://www.efsa.europa.eu/EFSA/ScientificPanels/DATEX/efsa_locale-1178620753812_ConciseEuropeanConsumptionDatabase.htm).

Ejaz AA et al. (2007). Could uric acid have a role in acute renal failure? *Clinical Journal of the American Society of Nephrology (CJASN)*, 2: 16–21.

EU (2001). Council Regulation EEC 2377/90. *Official Journal of the European Communities*, L 224 18.8.90 p. 1, amended M86, 2001, *Official Journal of the European Communities*, L 195 20.01.01, p. 32.

Fanelli GM, Beyer KH (1974). Uric acid in non-human primates with special reference to its renal transport. *Annual Reviews in Pharmacology*, 14: 355–364.

FAO (2007a). *Evaluations—Cyromazine (169)*. Rome, Food and Agriculture Organization of the United Nations, Joint FAO/WHO Meeting on Pesticide Residues (http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/2007_eva/Cyromazine.pdf).

FAO (2007b). Cyromazine (169). In: *Pesticide residues in food—2007. Report 2007*. Rome, Food and Agriculture Organization of the United Nations, Joint FAO/WHO Meeting on Pesticide Residues, pp. 98–100 (FAO Plant Production and Protection Paper 191; ftp://ftp.fao.org/docrep/fao/010/a1556e/a1556e00.pdf).

Fathallah-Shaykh F, Neiberger R (2008). Uric acid stones. *E-medicine* (http://emedicine.medscape.com/article/983759-overview; updated 8 July 2008, accessed 2 December 2008).

Fiechtner JJ, Simkin PA (1980). Monosodium urate monohydrate as spherulites. *Advances in Experimental Medicine and Biology*, 122A: 141–143.

Fiechtner JJ, Simkin PA (1981). Urate spherulites in gouty synovia. *JAMA: the Journal of the American Medical Association*, 245: 1533–1536.

Filigenzi MS et al. (2008). Diagnostic determination of melamine and related compounds in kidney tissue by liquid chromatography/tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*, 56(17): 7593–7599.

FSANZ (2008). *Melamine in foods from China*. Food Standards Australia New Zealand (http://www.foodstandards.gov.au/newsroom/factsheets/factsheets2008/melamineinfoodsfromchina/index.cfm; updated 29 October 2008).

Guan N et al. (2009). Melamine-contaminated powdered formula and urolithiasis in young children. *New England Journal of Medicine*, 4 February [Epub ahead of print].

Hammond BG et al. (1986). A review of toxicology studies on cyanurate and its chlorinated derivatives. *Environmental Health Perspectives*, 69: 287–292.

Health Canada (2008a). *Survey and health risk assessment of background levels of melamine in infant formula allowed for sale in Canada*. Ottawa, Ontario, Health Canada, Health Products and Food Branch, Food Directorate, Bureau of Chemical Safety, November 2008 (http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/pubs/melamine_survey-enquete_hra-ers-eng.pdf).

Health Canada (2008b). *Health Canada's risk assessment for melamine in foods containing milk and milk-derived ingredients*. Ottawa, Ontario, Health Canada (summary only available at http://www.hc-sc.gc.ca/fn-an/securit/chem-chim/melamine-eng.php; accessed 13 November 2008).

Heck HD'A, Tyl RW (1985). The induction of bladder stones by terephthalic acid, dimethyl terephthalate, and melamine (2,4,6-triamino-s-triazine) and its relevance to risk assessment. *Regulatory Toxicology and Pharmacology*, 5: 294–313.

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).

Hendry JA, Rose FL, Walpole AL (1951). Cytotoxic agents: 1, methylolamides with tumour inhibitory activity, and related inactive compounds. *British Journal of Pharmacology and Chemotherapy*, 6: 201–234.

HKCFS (2008). *Melamine in Mainland's milk powder incident*. Government of the Hong Kong Special Administrative Region, Centre for Food Safety (http://www.cfs.gov.hk/english/whatsnew/whatsnew_fstr/whatsnew_fstr_Melamine_in_Mainland_Milk_Powder_Incident.html).

Hodge HC et al. (1965). Toxicity of sodium cyanurate. *Toxicology and Applied Pharmacology*, 7: 667–674.

IARC (1999). Melamine. In: *Some chemicals that cause tumours of the kidney or urinary bladder in rodents, and some other substances*. Lyon, International Agency for Research on Cancer (IARC Monographs, Vol. 73).

INFOSAN (2008). *Melamine-contaminated products, China.* International Food Safety Authorities Network, 10 November 2008 (Emergency Alert Update No. 11).

Institut National de Santé Publique du Québec (2001). *A practical guide to baby care*. Quebec City, Quebec, Government of Quebec.

IPCS (1987). *Principles for the safety assessment of food additives and contaminants in food*. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 70).

Ishiwata H, Inoue T, Tanimura A (1986). Migration of melamine and formaldehyde from tableware made of melamine resin. *Food Additives and Contaminants*, 3(1): 63–70.

Ishiwata H et al. (1987). Liquid chromatographic determination of melamine in beverages. *Journal of the Association of Official Analytical Chemists*, 70(3): 457–460.

IUCLID (2000). *International Uniform Chemical Information Database*. European Commission (http://iuclid.echa.europa.eu/).

JAVMA (2007). Melamine adulterates component of pellet feeds. *Journal of the American Veterinary Medical Association*, 231(1): 17.

Johnson WJ, Stavric B, Chartrand A (1969). Uricase inhibition in the rat by s-triazines: An animal model for hyperuricemia and hyperuricosuria. *Proceedings of the Society for Experimental Biology and Medicine*, 131: 8–12.

Jutzi K, Cook AM, Hutter R (1982). The degradative pathway of the s-triazine melamine. The steps to ring cleavage. *Biochemistry Journal*, 208: 679–684.

Karbiwnyk CM et al. (2008). Determination of cyanuric acid residues in catfish, trout, tilapia, salmon and shrimp by liquid chromatography–tandem mass spectrometry. *Analytica Chimica Acta*, in press (available online 8 September 2008; doi:10.1016/j.aca.2008.08.037).

Karras G et al. (2007). Fate of cyromazine applied in nutrient solution to a gerbera (*Gerbera jamesonii*) crop grown in a closed hydroponic system. *Crop Protection*, 26: 721–728.

Kenney IJ (1991). Renal sonography in long standing Lesch-Nyhan syndrome. *Clinical Radiology*, 43(1): 39–41.

Kim YB et al. (2008). *Worsening nephrotoxicity by combination of melamine and cyanuric acid in rats.* Unpublished report. Cheongju Chungbuk, Republic of Korea, Chungbuk National University, College of Veterinary Medicine.

Korea Food and Drug Administration (2007). Monitoring on phenol, formaldehyde and melamine in utensils, containers and packaging. *Annual Report of the Korea Food and Drug Administration.* Vol. 11.

Kuo H-S, Kang J-J (2008). *Melamine-tainted non-dairy product investigation report. Surveillance report on melamine contamination in Taiwan.* Taiwan, China, Institute of Toxicology, College of Medicine, National Taiwan University; Bureau of Food and Drug Analysis, Department of Health; Centers of Disease Control, Department of Health; Taipei Hospital, Department of Health.

Lambert CC et al. (1998). Uric acid accumulation in the solitary ascidian *Corella inflata*. *Journal of Experimental Zoology*, 282: 323–331 (http://faculty.fullerton.edu/kkantardjieff/cvs/j_exper_zool_1998.pdf; accessed 19 September 2007).

Lauterburg B et al. (1977). The defect of uric acid metabolism in Eck-fistula rats. *Journal of Laboratory and Clinical Medicine*, 90: 92–100.

Lim LO et al. (1990). Disposition of cyromazine in plants under environmental conditions. *Journal of Agricultural and Food Chemistry*, 38: 860–864.

Lipschitz WL, Stokey E (1945). The mode of action of three new diuretics: Melamine, adenine and formoguanamine. *Journal of Pharmacology and Experimental Therapeutics*, 83: 235–249.

Losito A et al. (2009). Increased acid excretion in kidney stone formers with essential hypertension. *Nephrology, Dialysis, Transplantation*, 24(1): 137–141.

Luengyosluechakul S (2007). *Evidence of melamine and related substances contamination to animal feed in Thailand*. Bangkok, Thailand, Chulalongkorn University, Faculty of Veterinary Science, Department of Veterinary Medicine (http://www.vet.chula.ac.th/~tjvm/full_text/V37/v374/4_37_04.pdf; accessed 17 February 2009).

Lund KH, Petersen JH (2006). Migration of formaldehyde and melamine monomers from kitchen- and tableware made of melamine plastic. *Food Additives and Contaminants*, 23(9): 948–955.

MacKenzie HI (1966). Melamine for sheep. *Journal of the South African Veterinary Medical Association*, 37: 153–157.

MacKenzie HI, van Rensburg IBJ (1968). Ammelide and ammeline as nonprotein nitrogen supplements for sheep. *Journal of the South African Veterinary Medical Association*, 39: 41–45.

Mast RW, Naismith RW, Friedman MA (1982). Mouse micronucleus assay of melamine. *Environmental and Molecular Mutagenesis*, 4: 340–341 (Abstract No. Bi-8).

Mast RW et al. (1983). Metabolism, disposition and excretion of [^{14}C]melamine in male Fischer 344 rats. *Food and Chemical Toxicology*, 21: 807–810.

Matte D et al. (1990). [Kinetic study of *N*-chlorination of cyanuric acid in basic aqueous medium.] *Canadian Journal of Chemistry*, 68: 307–313 (in French).

Maxwell GR (2007). Synthetic nitrogen products. In: *Kent and Riegel's handbook of industrial chemistry and biotechnology*, 11th ed. New York, NY, Springer, pp. 996–1085.

Mazaev VT (1962). On the question of the content of cyanuric acid and its monovalent sodium salt in the water of reservoirs. *Gigiena i Sanitaria*, 27: 13–19.

Melnick RL et al. (1984). Urolithiasis and bladder carcinogenicity of melamine in rodents. *Toxicology and Applied Pharmacology*, 72: 292–303.

Mifflin MD et al. (1990). A new predictive equation for resting energy expenditure in healthy individuals. *American Journal of Nutrition*, 51: 241–247.

Muniz-Valencia R et al. (2008). Method development and validation for melamine and its derivatives in rice concentrates by liquid chromatography. Application to animal feed samples. *Analytical and Bioanalytical Chemistry*, 392(3): 523–531.

Negri AL et al. (2007). Clinical and biochemical profile of patients with "pure" uric acid nephrolithiasis compared with "pure" calcium oxalate stone formers. *Urological Research*, 35(5): 247–251.

NTP (1983). *Carcinogenesis bioassay of melamine (CAS No. 108-78-1) in F344/N rats and B6C3F1 mice (feed study)*. Research Triangle Park, NC, and Bethesda, MD, United States Department of Health and Human Services, Public Health Service, National Institutes of Health, National Toxicology Program (NTP Technical Report Series No. 245; NTP-81-86; NIH Publication No. 83-2501; http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr245.pdf).

OECD (1998). *Screening Information Dataset (SIDS) for melamine. CAS No. 108-78-1*. Paris, Organisation for Economic Co-operation and Development (http://www.chem.unep.ch/irptc/sids/OECDSIDS/108781.pdf).

OECD (1999). *Screening Information Dataset (SIDS) for isocyanuric acid. CAS No. 108-80-5*. Paris, Organisation for Economic Co-operation and Development (http://www.chem.unep.ch/irptc/sids/OECDSIDS/108805.pdf).

Ogasawara H et al. (1995). Urinary bladder carcinogenesis induced by melamine in F344 male rats: Correlation between carcinogenicity and urolith formation. *Carcinogenesis*, 16: 2773–2777.

Okumura M et al. (1992). Relationship between calculus formation and carcinogenesis in the urinary bladder of rats administered the non-genotoxic agents thymine or melamine. *Carcinogenesis*, 13(6): 1043–1045.

Ono S et al. (1998). Determination of melamine derivatives, melame, meleme, ammeline and ammelide by high-performance cation-exchange chromatography. *Journal of Chromatography A*, 815: 197–204.

Patakioutas D et al. (2007). Application and fate of cyromazine in a closed-cycle hydroponic cultivation of bean (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry*, 55: 9928–9935.

Puschner B et al. (2007). Assessment of melamine and cyanuric acid toxicity in cats. *Journal of Veterinary Diagnostic Investigation*, 19: 616–624.

RASFF (2008). *The Rapid Alert System for Food and Feed (RASFF), Annual Report, 2007*. European Commission, Directorate-General for Health and Consumers (http://ec.europa.eu/food/food/rapidalert/report2007_en.pdf).

Reimschuessel R (2008). *Pathology of combined melamine/cyanuric acid*. Presented at the China–US Workshops on Food Safety, Beijing, China, November 2008.

Reimschuessel R et al. (2008). Evaluation of the renal effects of experimental feeding of melamine and cyanuric acid to fish and pigs. *American Journal of Veterinary Research*, 69: 1217–1228.

Reyers F (2007). Melamine-contaminated pet food. The South African experience. *Veterinary News*, May issue, pp. 8–12.

Reyers F (2008). Melamine is back. In milk and in South Africa. A veterinary perspective. *Veterinary News*, December issue.

Root DS, Hongtrakul T, Dauterman WC (1996). Studies on the absorption, residues and metabolism of cyromazine in tomatoes. *Pesticide Science*, 48: 25–30.

Sancho JV et al. (2005). Residue determination of cyromazine and its metabolite melamine in chard samples by ion-pair liquid chromatography coupled to electrospray tandem mass spectrometry. *Analytica Chimica Acta*, 530: 237–243.

Schramek P, Heidenreich A, Engelmann UH (1993). Sex-dependent urolithiasis in the portacaval shunt rat. Part I. *Urology International*, 50(2): 77–81.

Seto CT, Whitesides GM (1990). Self-assembly based on the cyanuric acid–melamine lattice. *Journal of the American Chemical Society*, 112: 6409–6411.

Shelton DR et al. (1997). Metabolism of melamine by *Klebsiella terragena*. *Applied Environmental Microbiology*, 63: 2832–2835.

Sherrington DC, Taskinen KR (2001). Self-assembly in synthetic macromolecular systems via multiple hydrogen bonding interactions. *Chemical Society Reviews*, 30: 83–93 (DOI: 10.1039/b008033k).

Smoker MS, Krynitsky AJ (2008). *Interim method for determination of melamine and cyanuric acid residues in foods using LC-MS/MS: version 1.0*. Washington, DC, United States Food and Drug Administration, pp. 1–27 (Laboratory Information Bulletin No. 4422; http://www.cfsan.fda.gov/~frf/lib4422.html).

Stapleton FB (1983). Renal uric acid clearance in human neonates. *Journal of Pediatrics*, 103(2): 290–294.

Stavric B, Johnson WJ, Grice HC (1969). Uric acid nephropathy: An experimental model. *Proceedings of the Society for Experimental Biology and Medicine*, 130: 512–516.

Sugita T, Ishiwata J, Maekawa A (1991). Intestinal absorption and urinary excretion of melamine in male Wistar rats. *Journal of the Food Hygienic Society of Japan*, 32: 439–443.

Sun N et al. (2008). [Melamine related urinary calculus and acute renal failure in infants.] *Zhonghua Er Ke Za Zhi*, 46(11): 810–815 (in Chinese).

Teare JA, ed. (1999). *Physiological data reference values—1999*. Eagan, MN, International Species Information System.

Thompson ME et al. (2008). Characterization of melamine-containing and calcium oxalate crystals in three dogs with suspected pet food-induced nephrotoxicosis. *Veterinary Pathology*, 45: 417–426.

Tiu RV et al. (2007). Tumor lysis syndrome. *Seminars in Thrombosis and Hemostasis*, 33: 397–407.

Tolleson WH (2008). Renal toxicity of pet foods contaminated with melamine and related compounds. In: *Proceedings of the 235th National Meeting of the American Chemical Society, New Orleans, LA, 9 April 2008*. Washington, DC, American Chemical Society.

Turnipseed SB et al. (2008). Determination of melamine and cyanuric acid residues in infant formula using LC-MS/MS. Washington, DC, United States Food and Drug Administration, pp. 1–13 (Laboratory Information Bulletin, Vol. 24).

USEPA (1999). Cyromazine; pesticide tolerance. *Federal Register*, 64(178): 50043–50050.

USEPA (2007). Cyromazine: Registration review summary document. Washington, DC, United States Environmental Protection Agency (http://www.regulations.gov/fdmspublic/component/main?main=DocumentDetail&d=EPA-HQ-OPP-2006-0108-0003).

USFDA (2003a). *Code of Federal Regulations Title 21, Volume 3, Part 178 (Indirect Food Additives: Adjuvants, Production Aids, and Sanitizers), Section 178.1010 (Sanitizing Solutions)*. Washington, DC, United States Food and Drug Administration (http://www.cfsan.fda.gov/~lrd/fcf178.html).

USFDA (2003b). *Code of Federal Regulations Title 21, Chapter I, Part 573, Section 573.220, Feed-grade biuret*. Washington, DC, United States Food and Drug Administration (http://cfr.vlex.com/vid/19712997).

USFDA (2007). *Interim melamine and its analogues safety/risk assessment*. Washington, DC, United States Food and Drug Administration, Center for Food Safety and Applied Nutrition, 25 May 2007 (http://www.cfsan.fda.gov/~dms/melamra.html).

Watanabe S et al. (2002). Uric acid, hominoid evolution, and the pathogenesis of salt-sensitivity. *Hypertension*, 40: 355–360.

Weintraub PG (2001). Effects of cyromazine and abamectin on the pea leafminer *Liriomyza huidobrensis* (Diptera: Agromyzidae) and its parasitoid *Diglyphus isaea* (Hymenoptera: Eulophidae) in potatoes. *Crop Protection*, 20: 207–213.

Wheeler AG et al. (1985). Three generation reproduction study in rats administered cyanurate. *Toxicologist*, 5: 189.

Whitesides GM, Mathias JP, Seto CT (1991). Molecular self-assembly and nanochemistry: A chemical strategy for the synthesis of nanostructures. *Science*, 254: 1312–1319.

WHO (2004). Sodium dichloroisocyanurate. In: *Safety evaluation of certain food additives and contaminants*. Prepared by the sixty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Food Additives Series, No. 52; http://whqlibdoc.who.int/publications/2004/924166052X.pdf).

WHO (2007). *Sodium dichloroisocyanurate in drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality*. Geneva, World Health Organization (WHO/SDE/WSH/07.01/3; http://www.who.int/water_sanitation_health/dwq/chemicals/second_addendum_sodium_dichlorois ocyanurate.pdf).

WHO (2008a). Melamine-contaminated powdered infant formula, China. *Weekly Epidemiological Record*, No. 39. Geneva, World Health Organization, p. 350 (http://www.who.int/wer/2008/wer8339.pdf).

WHO (2008b). *Melamine-contamination event, China, September–October 2008*. Geneva, World Health Organization (http://www.who.int/foodsafety/fs_management/infosan_events/en/print.html; accessed 12 November 2008).

WHO (2009). *GEMS/Food consumption cluster diets*. Geneva, World Health Organization (http://www.who.int/foodsafety/chem/gems/en/index1.html).

Worzalla JF et al. (1974). Metabolism of hexamethylmelamine-ring-^{14}C in rats and man. *Cancer Research*, 34: 2669–2674.

Wu X et al. (1989). Urate oxidase: Primary structure and evolutionary implications. *Proceedings of the National Academy of Sciences of the United States of America*, 86: 9412–9416.

Wu X et al. (1994). Hyperuricemia and urate nephropathy in urate oxidase-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America*, 91: 742–746.

**Expert Meeting to Review Toxicological Aspects of
Melamine and Cyanuric Acid**

Ottawa, Canada, 1–4 December 2008

LIST OF PARTICIPANTS

Ms J. Baines, Food Composition, Evaluation and Modelling, Food Standards Australia New Zealand, Stirling, ACT, Australia (*Rapporteur*)

Dr J. Chen, Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, Beijing, China (*Chair*)

Mr S.J. Crossley, Food Safety and Nutrition (Europe), Exponent International Limited, Harrogate, England

Dr G.W. Diachenko,[1] United States Food and Drug Administration, College Park, Maryland, USA

Mr M. Feeley, Chemical Health Hazard Assessment Division, Bureau of Chemical Safety, Health Products and Food Branch, Health Canada, Ottawa, Ontario, Canada (*Rapporteur*)

Dr D.G. Hattan,[1] Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA (*Workgroup Chair*)

Ms C. Hilts, Bureau of Chemical Safety, Food Directorate, Health Canada, Ottawa, Ontario, Canada

Dr M. Hirose, Commissioner, Food Safety Commission, Tokyo, Japan

Dr B. Hoff, Animal Health Laboratory, Laboratory Services Division, University of Guelph, Guelph, Ontario, Canada (*Workgroup Chair*)

Dr S.-H. Jeong, Toxicology and Chemistry Division, National Veterinary Research and Quarantine Service, Ministry of Food, Agriculture, Forestry and Fisheries, Anyang City, Republic of Korea

Dr J.-J. Kang, Institute of Toxicology, College of Medicine, National Taiwan University, Taipei, Taiwan, China

Professor M.A. Mohd, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Dr M.J. Murphy, Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota, USA

---

[1] The opinions and information in this report are those of the authors and do not represent the views and/or policies of the United States Food and Drug Administration.

Mr L. Pelletier, Bureau of Chemical Safety, Food Directorate, Health Canada, Ottawa, Ontario, Canada

Dr B. Puschner, School of Veterinary Medicine, University of California, Davis, California, USA

Dr R. Reimschuessel,[1] Office of Research, Center for Veterinary Medicine, United States Food and Drug Administration, Laurel, Maryland, USA

Dr F. Reyers, Consultant Veterinary Pathologist, Garsfontein East, South Africa

Professor O. Sabzevari, Department of Toxicology and Pharmacology, Faculty of Pharmacy, University of Tehran Medical Sciences, Tehran, Iran

Dr S.K. Saxena, National Analytical Laboratory, National Dairy Development Board, Anand, Gujarat, India

Professor Y. Shen, Beijing Haemodialysis Centre for Children, Beijing, China

Dr S. Tittlemier, Food Research Division, Health Canada, Ottawa, Ontario, Canada

Dr Z. Wang, Department of Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China

Dr H.J. Yoon, Department of Food Evaluation, Korea Food and Drug Administration, Seoul, Republic of Korea

Secretariat

Ms D. Battaglia, Animal Production and Health Division, Agriculture and Consumer Protection Department, Food and Agriculture Organization of the United Nations, Rome, Italy

Ms R. Clarke, Food Quality and Standards Service, Nutrition and Consumer Protection Division, Food and Agriculture Organization of the United Nations, Rome, Italy

Dr S.B. Godefroy, Bureau of Chemical Safety, Food Directorate, Health Canada, Ottawa, Ontario, Canada

Dr J. Schlundt, Food Safety, Zoonoses and Foodborne Diseases, World Health Organization, Geneva, Switzerland

Ms M. Sheffer, WHO Editor, Ottawa, Ontario, Canada

Dr A. Tritscher, Department of Food Safety, Zoonoses and Foodborne Diseases, World Health Organization, Geneva, Switzerland

---

[1] The opinions and information in this report are those of the authors and do not represent the views and/or policies of the United States Food and Drug Administration.

**Expert Meeting to Review Toxicological Aspects of
Melamine and Cyanuric Acid**

In collaboration with FAO
Supported by Health Canada

Ottawa, Canada, 1–4 December 2008
Sir Frederick Banting Building

AGENDA

1. Opening of the meeting and welcome by:
   - Official of Health Canada
   - WHO and FAO
   - Introductory round
2. Election of the Chair and of the Rapporteur
3. Adoption of the agenda
4. Declarations of interests and confidentiality agreements
5. Brief introduction to the melamine event and purpose of the meeting
6. Discussion on the chemistry, analytical methods, occurrence and exposure assessment (working group)
7. Discussion on toxicology and risk assessment (working group)
8. Other topics
9. Adoption of executive summary and key conclusions and recommendations

LIST OF CONTRIBUTORS TO BACKGROUND DOCUMENTS

1. The chemistry of melamine alone and in combination with related compounds

W.H. Tolleson, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR, USA

G.W. Diachenko, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA

D. Folmer, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA

D. Doell, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA

D. Heller, Center for Veterinary Medicine, Food and Drug Administration, Laurel, MD, USA

2. Methods for the analysis of melamine and related compounds in foods and animal feeds

S. Tittlemier, Health Canada, Ottawa, Ontario, Canada

3. Occurrence of melamine in food and feeds

C. Hilts, Health Canada, Ottawa, Ontario, Canada

L. Pelletier, Health Canada, Ottawa, Ontario, Canada

4. Dietary exposure assessment

S.J. Crossley, Exponent International (Europe) and Exponent Inc.

B. Petersen, Exponent International (Europe) and Exponent Inc.

5. Melamine: Epidemiology

D. Lo-Fo-Wong, Department of Food Safety, Zoonoses and Foodborne Diseases, World Health Organization, Geneva, Switzerland

S. Hird, Department of Food Safety, Zoonoses and Foodborne Diseases, World Health Organization, Geneva, Switzerland

6. Toxicology of melamine and its analogues

R. Reimschuessel, Center for Veterinary Medicine, Food and Drug Administration, Laurel, MD, USA

D.G. Hattan, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA

Y. Gu, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA

7. Dose–response considerations: Benchmark dose analysis of melamine data in laboratory animals

C. Portier, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC, USA
F. Parham, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC, USA

LIST OF ABBREVIATIONS

ADI	acceptable daily intake
BMD	benchmark dose
BMD_{05}	benchmark dose for a 5% risk
BMD_{10}	benchmark dose for a 10% risk
BMDL	lower limit on the benchmark dose
$BMDL_{05}$	lower limit on the benchmark dose for a 5% risk
$BMDL_{10}$	lower limit on the benchmark dose for a 10% risk
CAS	Chemical Abstracts Service
CFR	Code of Federal Regulations (USA)
CYA	cyanuric acid
DAD	diode array detection
DNA	deoxyribonucleic acid
EFSA	European Food Safety Authority
ELISA	enzyme-linked immunosorbent assay
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GC	gas chromatography
GEMS/Food	Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme
HPLC	high-performance liquid chromatography
INFOSAN	International Food Safety Authorities Network
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
K_d	dissociation constant
LC	liquid chromatography
LD_{50}	median lethal dose
MEL	melamine
MRL	maximum residue limit
MS	mass spectrometry
MS/MS	tandem mass spectrometry
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program (USA)
SAR	Special Administrative Region
STMR	supervised trial median residue
TDI	tolerable daily intake
USA	United States of America
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
UV	ultraviolet
WHO	World Health Organization

DOSE CONVERSION TABLE

Where accurate doses cannot be calculated on the basis of measured body weights and food consumption, approximate doses can be estimated using the dose conversion factors in the following table, taken from IPCS (1987).

Table A5-1. Approximate relation of mg/kg in the diet to mg/kg body weight per day

Species	Weight (kg)	Food consumed per day (g) (liquids omitted)	Type of diet	1 mg/kg in food = x mg/kg body weight per day	1 mg/kg body weight per day = x mg/kg of diet
Mouse	0.02	3	Dry laboratory chow diets	0.150	7
Chick	0.40	50		0.125	8
Rat (young)	0.10	10		0.100	10
Rat (old)	0.40	20		0.050	20
Guinea-pig	0.75	30		0.040	25
Rabbit	2.0	60		0.030	33
Dog	10.0	250		0.025	40
Cat	2	100	Moist, semi-solid diets	0.050	20
Monkey	5	250		0.050	20
Dog	10	750		0.075	13
Human	60	1500		0.025	40
Pig or sheep	60	2400	Relatively dry grain forage mixtures	0.040	25
Cow (maintenance)	500	7500		0.015	65
Cow (fattening)	500	15 000		0.030	33
Horse	500	10 000		0.020	50