## SCIENTIFIC OPINION

# Scientific Opinion on Melamine in Food and Feed[1]

**EFSA Panel on Contaminants in the Food Chain (CONTAM) and EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)**[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 16 April 2010, replaces the earlier version published on 13 April 2010[4].

**ABSTRACT**

The European Food Safety Authority (EFSA) was asked by the European Commission to provide a scientific opinion related to the presence of melamine and the structural analogues (cyanuric acid, ammeline and ammelide) in food and feed. EFSA identified the potential sources of melamine and cyanuric acid in food that were not clearly related to incidences of adulteration, including food contact materials, and estimated the associated dietary exposure. Melamine does not exhibit systemic toxicity, but is able to complex with other substances such as endogenous uric acid or substances related to melamine to form crystals in the urine, which cause kidney damage. From the available toxicological data, a Tolerable Daily Intake (TDI) of 0.2 mg/kg body weight was established for melamine. Due to uncertainties in the exposure estimates, the human data related to adulteration in infant milk formula with melamine in 2008 were not considered to be sufficiently robust, to form the primary basis for the TDI, but provided supporting evidence for the TDI derived from animal studies. The exposure from background levels of melamine and cyanurate that can occur in food and feed from approved sources does not represent a risk to the human consumer or to animals. Exposure in children due to migration from food contact materials would be below or in the region of the TDI. The migration limit for melamine should be reconsidered in the light of the TDI taking into account all sources of exposure. The potential of melamine to form crystals is increased by concomitant exposure to cyanuric acid, and therefore the TDI is not appropriate for protection of consumer health in the presence of such concomitant exposure. This opinion does

---

1 On request from the European Commission, Question No EFSA-Q-2009-00234, adopted on 18 March 2010 by the CONTAM Panel and Question No EFSA-Q-2009-00235 adopted on 25 March 2010 by the CEF Panel.

2 CONTAM Panel members: Jan Alexander, Diane Benford, Alan Boobis, Sandra Ceccatelli, Jean-Pierre Cravedi, Alessandro Di Domenico, Daniel Doerge, Eugenia Dogliotti, Lutz Edler, Peter Farmer, Metka Filipič, Johanna Fink-Gremmels, Peter Fürst, Thierry Guerin, Helle Katrine Knutsen, Miroslav Machala, Antonio Mutti, Josef Schlatter and Rolaf van Leeuwen.

CEF Panel members: Arturo Anadon, Mona-Lise Binderup, Wilfried Bursch, Laurence Castle, Riccardo Crebelli, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Thomas Haertle, Trine Husøy, Klaus-Dieter Jany, Catherine Leclercq, Jean Claude Lhuguenot, Wim Mennes, Maria Rosaria Milana, Karla Pfaff, Kettil Svensson, Fidel Toldra, Rosemary Waring, Detlef Wölfle.

Correspondence: contam@efsa.europa.eu

3 Acknowledgement: The Panel wishes to thank the members of the Working Group on melamine in food for the preparation of this opinion: David Bell (December 2009), Diane Benford, Laurence Castle, Daniel Doerge, Lutz Edler, Johanna Fink-Gremmels, Helle Katrine Knutsen, Wim Mennes and EFSA's staff members Davide Arcella, Jean Lou Dorne, Marc Vandenbroeck, and Francesco Vernazza for the support provided to this EFSA scientific output.

4 The document was reformatted with minor editorial changes. David Bell is no longer a CEF Panel member since December 2009. Hence his name was removed from the list.

Suggested citation: EFSA Panel on Contaminants in the Food Chain (CONTAM) and EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Melamine in Food and Feed. EFSA Journal 2010; 8(4):1573. [145 pp.]. doi:10.2903/j.efsa.2010.1573. Available online: www.efsa.europa.eu

not consider the potential exposure to melamine and/or cyanurate that can arise from adulteration with these substances.

**KEY WORDS**

Melamine (CAS No 108-78-1), cyanuric acid (CAS No. 108-80-5), food, feed, occurrence, risk assessment, toxicity, tolerable daily intake (TDI).

**SUMMARY**

The European Food Safety Authority (EFSA) was asked by the European Commission (EC) to provide a scientific opinion related to the presence of melamine and the structural analogues such as cyanuric acid, ammeline and ammelide in food and feed.

Melamine (2,4,6-triamino-1,3,5-triazine, CAS No. 108-78-1) is produced as a high volume chemical. It can be present in food as a result of uses in food contact materials, including articles made of melamine-formaldehyde plastics, can coatings, paper and board and adhesives. The current specific migration limit (SML) laid down in European Union legislation for plastics is 30 mg/kg food. Melamine may also occur in food as a metabolite and degradation product of cyromazine, which is used as a plant protection product and as a veterinary drug, and potentially as a result of its use as a flame retardant. Depending on the purification process, melamine may contain different levels of the structurally related substances cyanuric acid, ammeline and ammelide. Cyanuric acid residues can also occur in food as a result of use of dichloroisocyanurates as a source of active chlorine in disinfection agents. Melamine and cyanuric acid can be present as impurities in urea-based feed for ruminants.

Illegal adulteration of food and feed with melamine has resulted in illness and deaths of human infants and pet animals (cats and dogs), primarily as a result of kidney damage caused by crystals or stones in the urinary tract. The pets were given feed adulterated with crude ("scrap") melamine also containing its analogues, and the crystals consisted of complexes of melamine with cyanuric acid. In the human infants, who were given infant formula adulterated with a relatively pure preparation of melamine, the crystals consisted of complexes of melamine with uric acid which occurs naturally in urine. Crystals have also been reported in livestock given feed contaminated with melamine and in experimental animals dosed with melamine either alone or together with cyanuric acid.

Following the incidents of adulterated food and feed, several analytical methods for the determination of melamine (with and without analogues) in various matrices have been developed. As a result, reliable extraction and sample clean-up techniques are available for most food types including foods high in protein, fat and carbohydrates. The most sensitive and selective analytical method to measure melamine and its structural analogues is LC-MS/MS (liquid chromatography coupled to tandem mass spectrometry).

EFSA received 2239 data on melamine occurence in food and feed from European countries. These were the results of targeted sampling focussed on products where adulteration was considered likely. Because they were not representative of background levels they were not considered appropriate for the assessment of background dietary exposure to melamine, as required by the terms of reference of this opinion. No data on cyanuric acid were provided by European countries. Data on melamine and cyanuric acid in food and feed were also submitted by the industry. The data on food were used by the Panel on Contaminants in the Food Chain (CONTAM Panel) as the basis for dietary exposure assessment, after excluding a small number of samples with high values that were related to incidents of adulteration. For adult high consumers, the dietary exposure estimates to melamine in different European Union (EU) countries based on the upper bound occurrence values is below 11 µg/kg body weight (b.w.) per day. For infants fed solely on formula, the dietary exposure estimates to melamine are all below 2 µg/kg b.w. per day. For adult high consumers, the dietary exposure estimates to cyanurate in different EU countries based on the upper bound occurrence values is below 16 µg/kg b.w. per day. For infants fed solely on formula, the dietary exposure estimates to cyanurate are all below 6 µg/kg b.w. per day. These estimates are considered to be conservative, because many of the occurrence data were upper bound values for samples with melamine or cyanuric acid below the limit of detection.

Melamine exposure resulting from the migration of food contact materials was estimated by the Panel on food contact materials, enzymes, flavourings and processing aids (CEF Panel). Preliminary rough estimates indicated that the highest potential exposure was in children. Therefore, melamine exposure resulting from the migration of food contact materials was estimated according to two more refined

scenarios (A and B), using the database on individual food consumption data for children" (EXPOCHI) for 13 different Member States and typical and high migration levels of melamine from a "standard" melaware article under typical (Scenario A) and severe time and temperature conditions (Scenario B), respectively. Exposure was calculated assuming that any food or beverage could come into contact with high melamine-releasing melaware articles and obtained by summing up, within each day, the exposure from all food groups for Scenario A. For scenario B only exposure from the one food item giving the highest exposure estimate within one day was considered. In this Scenario exposure to melamine from other food items was considered not to occur within the same day. Total exposure to melamine in one day considering typical migration levels (scenario A) ranged from 30 to 80 µg/kg b.w per day (mean) and from 50 to 120 µg/kg b.w per day (95$^{th}$ percentile). Exposure in one day from one food/beverage considering high migration levels (scenario B) ranged from 40 to 110 µg/kg b.w per day (mean) and from 70 to 230 µg/kg b.w per day (95$^{th}$ percentile).

Animal exposure to melamine was estimated for livestock based on the European action level of 2.5 mg/kg in mixed feed and in premixes, with lower and higher scenarios of 0.5 and 10 mg/kg, a range encompassing the data submitted by industry. In ruminants, dairy cows and dairy goats would be the most exposed to melamine (40 and 42 µg/kg b.w per day and160 and 169 µg/kg b.w per day if compound feed contained 2.5 mg/kg or 10 mg/kg melamine respectively). In monogastric livestock, poultry (broilers and laying hens) would be the most exposed to melamine (179 and 151 µg/kg b.w per day and 714 and 605 µg/kg b.w per day respectively if feed contained 2.5 or 10 mg/kg melamine respectively). In pet animals, exposure was estimated using scenarios based on melamine concentrations of 0.5, 2.5 and 10 mg/kg, and consumption based on protein requirements in canned food (average: 48g/kg) or dried food (average: 13.5g/kg). Exposure in cats and dogs would be 120 µg/kg b.w per day from canned food and 63 µg/kg b.w per day from dried food containing 2.5 mg/kg melamine. At 10 mg/kg melamine, exposure would be 480 µg/kg b.w per day from canned food and 250 µg/kg b.w per day from dried food. Exposure to cyanurate in ruminants has been estimated from a urea-based feed additive product defining levels of cyanurate up to 200 mg/kg and assuming the use of 30 g as per 100 kg animal, giving a daily intake of 60 µg/kg b.w per day mg/kg b.w of cyanurate.

Melamine is rapidly absorbed from the gastrointestinal tract and rapidly excreted from the body, with a half life of 4-5 hours in the rat and rhesus monkey, and little or no metabolism. The limited information available for cyanuric acid also indicates rapid absorption and rapid elimination via the urine with little or no biotransformation. If the melamine concentration in urine is sufficient to form crystals it causes proximal tubular damage in the kidney. Formation of the crystals is highly pH dependent and most likely to occur at about pH 5.5. Humans may be more susceptible to co-precipitation of melamine with uric acid because humans excrete more uric acid in the urine than most mammals owing to a lack of the enzyme urate oxidase, and also because the urinary pH is lower than that of rodents. In neonates, the excretion of uric acid in the urine is higher than in adults. Because it is the presence of the crystals in urine that results in kidney damage, the effects of melamine are not due to systemic toxicity but to its physicochemical properties.

The limited data available in livestock have indicated no effects of feeding melamine, 400 mg/kg b.w. per day in pigs and 456 mg/kg b.w. per day in fish and 180 mg/kg b.w. per day in cats. No other data on melamine toxicity alone were available in livestock, fish or pets. For cyanuric acid no adverse effects were seen in sheep at 600 mg/kg b.w. per day for 77 days, or at 400 mg/kg b.w. per day in pigs and 390 mg/kg b.w. per day in fish for 3 days. The estimated exposures to melamine and cyanuric acid at the scenarios of 0.5, 2.5 and 10 mg/kg in feed are well below these doses and are not expected to pose a risk to the animals.

Ammelide was not acutely toxic and did not induce sub-chronic toxicity at 372 mg/kg b.w per day when fed to sheep for 6 weeks. An average daily intake of 296 mg ammeline/kg b.w. per day and 97 mg/kg b.w. of a mixture of ammeline and ammelide respectively in the diet of sheep caused the death of half of the animals. No other data on ammeline or ammelide toxicity were available for livestock, fish and pets.

In laboratory animals, melamine has low acute toxicity. Repeat dosing resulted in lesions in the kidney and urinary tract associated with the formation of urinary crystals. A 13-week study with dietary exposure of male rats to melamine provided the best basis for characterising the dose-response relationship in experimental animals. The Panel identified, for a 10 % increase in urinary bladder crystals, a benchmark dose ($BMD_{10}$) of 41 mg/kg b.w. per day and its lower confidence limit ($BMDL_{10}$) of 19 mg/kg b.w. per day. The Panel considered the factors that could contribute to inter- and intra-species differences in the effects of melamine, such as the impact of urinary uric acid concentrations and pH on the formation of the melamine complexes with uric acid, and concluded that the default uncertainty factor of 100 was appropriate for deriving a tolerable daily intake (TDI). The Panel concluded that the TDI set by Scientific Committee for Food is no longer appropriate. The Panel established a TDI of 0.2 mg/kg b.w. by dividing the $BMDL_{10}$ of 19 mg/kg b.w. per day by the uncertainty factor of 100, with rounding to a single significant figure. This TDI is considered appropriate for infants, except for those born prematurely who have higher urinary uric acid levels and greater immaturity of kidney function.

From the human data, the CONTAM Panel calculated a $BMD_{10}$ of 1.1 mg/kg b.w. per day and a $BMDL_{10}$ of 0.74 mg/kg b.w. per day for a 10 % increased incidence of nephrolithiasis. Whilst these data were not considered suitable for deriving a TDI due to uncertainties in the exposure assessment, this human $BMDL_{10}$ provides supporting evidence for the adequacy of the TDI of 0.2 mg/kg b.w. derived from animal data.

A TDI for cyanuric acid of 1.3 mg/kg b.w. was set based on a previous evaluation of the disinfectant dichloroisocyanurate. The toxicological databases for ammelide and ammeline are extremely limited and hence no TDI could be established.

Co-exposure to melamine and cyanuric acid in livestock, fish, pets and laboratory animals showed higher toxicity compared with melamine or cyanuric acid alone, due to the renal crystal formation. There is limited evidence that ammelide and ammeline can also form crystals with melamine. The currently available information does not allow identification of a factor by which the toxicity is increased by co-exposure. Therefore the TDI for melamine is not appplicable if there is significant concomitant exposure to cyanuric acid, ammelide or ammeline due to the increased potential for formation of urinary crystals.

Dietary exposure to melamine and cyanuric acid individually estimated from the available data relating to background sources is well below the respective TDIs and does not raise concerns for the health of consumers or animals. For food contact materials, estimated acute exposures in children to melamine from melaware were also below the TDI with the exception of the conservative assessment ($95^{th}$ percentile) for children, who might be exposed to melamine levels slightly above the TDI. For these exposure estimates a health concern is not identified, due to their conservative nature.

It is recommended that the current SML for melamine from food contact materials is reconsidered in the light of the TDI of 0.2 mg/kg b.w. taking into account all sources of exposure. There is a need for data on co-occurrence of melamine and its structural analogues (cyanuric acid, ammelide, ammeline) in food or feed. There is a need for additional information on the dose response relationships for combinations of melamine and and its structural analogues (cyanuric acid, ammelide, ammeline). Development of a physiologically-based toxicokinetic toxicodynamic model is also needed to improve the dose response modelling.

**TABLE OF CONTENTS**

**BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION**

## I. THE CONTAMINATION INCIDENTS

### I.1. The contamination incident in 2007

Since February 2007, unusual sickness and death of pet animals (cats and dogs) was reported in the United States (US). Following these reports an investigation was undertaken by the US authorities to trace the source of these animal health problems. It was found that wheat gluten originating from China and used for the production of pet food was at the origin of the animal health problems. Recall was initiated of pet food in which the wheat gluten was used.

Early in April 2007, the fraudulent addition of melamine, an industrial chemical used in plastics, glues, etc…, to wheat gluten imported from China, was found to be the cause of the animal health incidents. Later, melamine and cyanuric acid, a compound structurally related to melamine, were also found in rice protein concentrate imported from China. The levels of melamine found in wheat gluten and rice protein concentrate were in the range of 0.2 to 8 % (i.e. 2 to 80 grammes per kg).

As the protein concentration is measured by analysis of the nitrogen, the fraudulent addition of melamine (C3H6N6), a chemical substance rich in nitrogen, aims at enhancing the apparent protein content of wheat gluten and other protein sources. It appears that it is the combination of melamine and cyanuric acid, forming crystals in the kidney of animals, that has caused the animal health problems.

Member States were asked by the Commission on 2 May 2007 to control consignments of wheat gluten, corn gluten, corn meal, soy protein, rice bran and rice protein concentrate originating from third countries, in particular from China, for the presence of melamine and related compounds.

The Commission has also sent a request to EFSA on 8 May 2007 to obtain an urgent opinion on the risks for animal health and public health of the presence of melamine and structurally related compounds in feed and food. EFSA has issued on 8 June 2007 a statement on the issue[5].

Taking into account the conclusions of the EFSA statement, the Member States agreed at the Standing Committee on the Food Chain and Animal Health, section Animal Nutrition, on 8 June 2007 on a harmonised enforcement approach in case of a finding of presence of melamine and related compound (ammeline, ammelide, cyanuric acid) in feed stuffs[6].

Taking into account the results of the controls and the measures taken and commitment by the Chinese authorities, it was agreed at the meeting of the Committee on 22 October 2007 that there was no longer a need to maintain an increased frequency of import controls at import for the presence of melamine and related compounds in protein-rich feed ingredients and an 'at random' official import control regime was from then onwards sufficient and appropriate[7].

### I.2. The contamination incident in 2008

In the second week of September 2008, the Commission was made aware that high levels of melamine were found in infant milk and other milk products in China. Melamine is a chemical intermediate used

[5] 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. (Question n° EFSA-Q-2007-093). Available at: http://www.efsa.europa.eu/cs/BlobServer/Statement/efsa_statement_melamine_en_rev1,0.pdf?ssbinary=true

[6] Summary report of the meeting of Standing committee on the food Chain and Animal Health, section Animal Nutrition on 8 June 2007 available at: http://ec.europa.eu/food/committees/regulatory/scfcah/animalnutrition/summary07062007_en.pdf

[7] Summary report of the meeting of Standing committee on the food Chain and Animal Health, section Animal Nutrition on 22 October 2007 available at: http://ec.europa.eu/food/committees/regulatory/scfcah/animalnutrition/sum_22102007_en.pdf

in the manufacture of resins and plastics. Melamine, which is high in nitrogen, has been fraudulently added infant milk and milk to give the appearance of increased protein levels.

The high levels of melamine in infant milk resulted in China in very severe health effects in infants and young children. At least 6 children have died in China from severe kidney failure due to the melamine added to milk powder, and more than 200.000 infants and young children have been affected by kidney problems with more than 50.000 infants and young children hospitalized.

Imports of milk and milk products, including milk powder, originating from China have never been allowed into the Community. However, composite products such as chocolate, bonbons, biscuits containing milk or milk products are imported from China into the EU certain composite products could have been imported without undergoing systematic border checks. It could furthermore not be excluded that special import channels for such products exist (intended for instance for Chinese food shops).

To assess the risks related to the presence of melamine in composite products containing milk and milk products, such as chocolate, biscuits etc, at the request of the Commission the European Food Safety Authority (EFSA) issued on 24 September a scientific statement[8], which concludes that the highest risk would be represented by a worst case scenario according to which children with high daily consumption of biscuits and chocolates containing the highest proportion of milk powder with a contamination equal to the highest level found in milk powder from China. Melamine can be present in foods at low background levels following e.g. migration from food packaging material or as metabolite of the pesticide cyromazine.

A level of 2.5 mg melamine/kg was established as the appropriate level to distinguish between the unavoidable background presence of melamine (from food contact materials, pesticide use, etc.) and unacceptable adulteration. This level provides also a large margin of safety according to the EFSA statement on the risks of melamine in food. Methods of analysis with sufficient sensitivity exist to control the presence of melamine in food at levels of 2.5 mg/kg.

In order to protect public health, Commission Decision 2008/798/EC of 14 October 2008 imposing special conditions governing the import of products containing milk and milk products originating in or consigned from China and repealing Commission Decision 2008/757/EC[9] was adopted.

This Decision established protective measures for food and feed originating in or consigned from China such as:

- a prohibition of import into the Community of composite products containing milk and milk products intended for infants and young children.

- a physical control (sampling and analysis) on the presence of melamine of all consignments of composite products containing milk products, to be imported via specifically designated control points.

- an increased control on the presence of melamine on other feed and food products with a high protein content

Following the finding of high levels of melamine in soybean meal intended for feed and in ammonium bicarbonate, used as raising agent in food industry, Commission Decision 2008/921/EC of

[8] Statement of EFSA on risk for public health due to the presence of melamine in infant milk and other milk products in China (Question No EFSA-Q-2008-695). Available at: http://www.efsa.europa.eu/cs/BlobServer/Statement/contam_ej_807_melamine.pdf?ssbinary=true

[9] OJ L 273, 15.10.2008, p. 18.

9 December 2008 amending Decision 2008/798/EC[10] was adopted extending the safeguard measures to ammonium bicarbonate and to feed and food containing milk, milk products, soy and soy products.

## II. SCIENTIFIC ASSESSMENTS ON MELAMINE AND STRUCTURALLY RELATED COMPOUNDS

### II.1. Standing Committee For Food

The Scientific Committee for Food established at its meeting on 14 December 1984 a TDI for melamine of 0.5mg/kg b.w.[11]

### II. 2. EFSA statement related to melamine and structurally related compounds such as cyanuric acid in protein-rich ingredients used for feed and food

In this statement, issued on 7 June 2007, EFSA concluded the following:

The Scientific Committee for Food (SCF) derived a tolerable daily intake (TDI) of 0.5 mg/kg body weight (b.w.) per day for melamine for food contact materials but no details were given for its derivation. Recently the U.S. Food and Drug Administration (FDA) derived a TDI of 0.63 mg/kg b.w. per day which is in line with the TDI derived by the SCF. For melamine a specific migration limit of 30 mg/kg food was agreed by the SCF assuming a maximum consumption of 1 kg food containing the substance for a 60 kg person.

Based on the NOAEL of 154 mg/kg b.w. per day for sodium cyanurate derived from a 2-year study in rats, a TDI for sodium cyanurate of 1.5 mg/kg b.w. per day can be proposed using an uncertainty factor of 100.

There is a lack of toxicity data for ammeline and ammelide. Because of the structural similarities to melamine these compounds have been assumed to be of equal potency.

In conclusion, EFSA provisionally recommends to apply a TDI of 0.5 mg/kg b.w. per day for the total of melamine and its analogues (ammeline, ammelide, cyanuric acid).

Because of a lack of toxicity data in domestic animals, EFSA provisionally recommends to apply this tolerable intake level as established for humans also to domestic animals.

A source of uncertainty is the combined toxicity of melamine and cyanuric acid and their possible synergistic effects in relation to the recently observed toxicity linked to the acute renal failure and death of pet animals (cats and dogs) in the U.S. This mechanism is currently under investigation.

Occurrence data on melamine and its analogues in food and feed from Europe are needed to perform a comprehensive risk assessment.

### II.3. EFSA statement on risks for public health due to the presence of melamine in infant milk and other milk products in China

In this statement, issued on 24 September 2008, EFSA concluded the following:

EFSA was asked to consider health effects due to melamine exposure via the consumption of contaminated biscuits and confectionary.

[10] OJ L 331, 10.12.2008, p. 19.

[11] Report of the Scientific Committee for Food on certain monomers and other starting substances to be used in the manufacture of plastic materials and articles intended to come into contact with foodstuffs (opinion expressed on 14 December 1984), Reports of the Scientific Committee for Food, Seventeenth series, EUR 10778 EN, http://ec.europa.eu/food/fs/sc/*scf*/reports/scf_reports_17.pdf

The primary target organ for melamine toxicity is the kidney. There is uncertainty with respect to the time scale for the development of kidney damage. Thus, EFSA applied a tolerable daily intake (TDI) of 0.5 mg/kg body weight (b.w.) in considering possible health effects which might occur with repeated consumption of melamine contaminated products over a relatively short period.

Based on different exposure scenarios, estimated exposure does not raise concerns for the health of adults in Europe should they consume chocolates and biscuits containing contaminated milk powder. Children with a mean consumption of biscuits, milk toffee and chocolate made with such milk powder would not exceed the tolerable daily intake (TDI). However, in worst case scenarios with the highest level of contamination, children with high daily consumption of milk toffee, chocolate or biscuits containing high levels of milk powder would exceed the TDI. Children who consume both such biscuits and chocolate could potentially exceed the TDI by more than threefold. However, EFSA noted that it is presently unknown whether such high level exposure scenarios may occur in Europe.

### II.4. WHO Expert Meeting in collaboration with FAO on toxicological and health aspects of melamine and cyanuric acid, held from 1 to 4 December 2008 in Ottawa, Canada[12]

The WHO Expert Meeting came to the following conclusions:

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.

## III. SPECIFIC BACKGROUND INFORMATION

The Scientific Committee for Food has established for melamine a tolerable daily intake (TDI) of 0.5 mg/kg bodyweight (b.w.) in 1984 (see point II.1). In June 2007 EFSA recommended applying the TDI of 0.5 mg/kg b.w. also for the contamination case in pet food considering the data available on toxicity of melamine (see point II.2). In its statement of 24 September 2008 EFSA applied a TDI of 0.5 mg/kg b.w. in considering possible health effects which might occur with repeated consumption of melamine contaminated products over a relatively short period (see point II.3).

The WHO Expert Meeting reviewed the toxicological and health aspects of melamine and cyanuric acid established for melamine the application of a TDI of 0.2 mg/kg b.w (see point II.4).

It is appropriate for EFSA to re-assess the TDI of 0.5 mg/kg bodyweight in view of the outcome of the WHO Expert Meeting.

In EU legislation[13], a Specific Migration Limit (SML) for melamine has been established. Melamine is used in melamine ware/plastics used as food contact materials and can migrate from the food contact material in the food. This SML is derived from the TDI for melamine, i.e. 0.5 mg/kg, whereby it is assumed in a very worst case scenario that a person can be exposed daily to 1 kg of food which has

[12] The full report of the WHO Expert Meeting in collaboration with FAO on Toxicological and Health Aspects of Melamine and Cyanuric Acid is available at: http://whqlibdoc.who.int/publications/2009/9789241597951_eng.pdf

[13] Commission Directive 2002/72/EC of 6 August 2002 relating to plastic materials and articles intended to come into contact with foodstuffs (OJ L 220, 15.8.2002, p. 18).

been in contact with a food contact material containing melamine. This SML serves as criterion to evaluate if a certain substance may be used in food contact material. Experimental tests are performed with food simulants under worst case conditions in order to verify if the migration is not larger than the SML. In case the migration under these conditions is not higher than the SML, the substance may be authorised for use in food contact materials. However it appears from available data that in reality, migration of melamine from food contact materials would not be more than a few mg/kg food.

At the 3rd Session of the Codex Committee on Contaminants in Foods, held in Rotterdam, the Netherlands from 23 to 27 March 2009[14], it was agreed to initiate new work on the setting of maximum levels for melamine in feed and food. It is not yet foreseen to consider maximum levels for melamine-related chemicals e.g. cyanuric acid, ammelide and ammeline, but it was recognised that these chemicals present, in combination with melamine, a more unique toxicological concern compared to melamine alone. At a later stage, information on this co-occurrence might result in the setting of new maximum levels and/or revision of the maximum levels based on the toxicity of melamine alone.

Data have shown carry-over from feed to products of animal origin (e.g. milk, meat) including fish.

## TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Art. 29 (1) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide a scientific opinion related to the presence of melamine and the structural analogues such as cyanuric acid, ammeline and ammelide in food and feed.

This scientific opinion should comprise the

- re-assessment of the TDI on melamine in view of the outcome of the WHO Expert meeting.

- determination of the exposure levels of melamine for the different animal species (difference in sensitivity between animal species) above which adverse effects on animal health can be observed (NOAEL/LOAEL)

- determination of the level of transfer/carry-over of melamine from the feed to the products of animal origin, either as melamine or as a metabolite of melamine.

- identification of the different sources of the background presence of melamine and the structural analogues cyanuric acid, ammelide and ammeline in feed and food other than adulteration or misuse.

- assessment of the exposure to melamine of different animal species taking into background presence of melamine in feed

- human exposure assessment to melamine taking into the background presence of melamine in food, including in food of animal origin following carry over from feed. Also the exposure of vulnerable groups of the population as well of specific groups of consumers who might be exposed to higher levels of melamine than average as the consequences of their dietary habits should be addressed.

- assessment of the possible risks for animal health related to the background presence of melamine in feed.

- assessment of the possible risks for public health related to the background presence of melamine in food.

[14] Report available at http://www.codexalimentarius.net/download/report/722/al32_41e.pdf

- in addition and insofar data are available, assessment of the risks for animal and public health following co-exposure of animals and humans to melamine and structurally related compounds such as cyanuric acid, ammelide and ammeline.

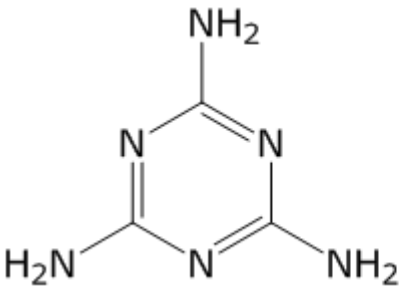
## APPROACH TAKEN TO ANSWER THE TERMS OF REFERENCE

After having received this request from the European Commission, EFSA allocated the mandate to the Panel on Contaminants in the food chain (CONTAM Panel) and the Panel on food contact materials, enzymes, flavourings and processing aids (CEF Panel). The scientific opinion was adopted by the CONTAM Panel on 18 March 2010. Chapters 2.1.1. Specific migration limit for food contact materials, 3.1.1. Food contact materials and articles, 4.4.4. on Melamine levels in food and feed from food contact materials, and 5.1.2.5. Contributions of food contact materials to melamine dietary exposure, and their respective conclusions were adopted by CEF Panel on 25 March 2010. The CEF Panel also endorsed the chapters 6.6. Derivation of the tolerable daily intake(s) for melamine and cyanurate, and chapter 7.2. Human health risk assessment.

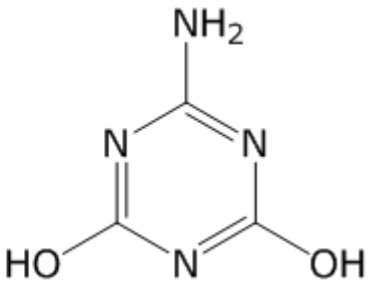
**ASSESSMENT**

## 1. Introduction

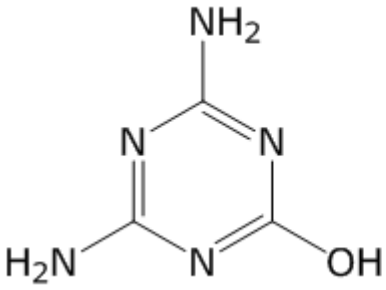
Melamine (2,4,6-triamino-1,3,5-triazine, CAS No. 108-78-1, molecular formula C3H6N6, molecular weight 126 g/mol) is produced in high volumes mainly for the synthesis of melamine-formaldehyde resins used in the manufacture of laminates, plastics and coatings, including food contact materials such as kitchenware. In addition, melamine and a number of related compounds are used as flame retardants. Melamine has also been used as a nitrogen source in animal nutrition and is a metabolite and degradation product of the pesticide and veterinary drug, cyromazine. Depending on the purification process, melamine may contain a number of structurally related by-products, particularly cyanuric acid, ammelide and ammeline (see structures in Figure 1). "Scrap melamine" is a residue from the melamine industry that in addition to melamine contains residual amounts of oxytriazines, including cyanuric acid (CAS No. 108-80-5)[15]



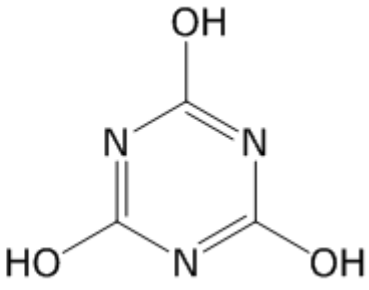

Melamine




Ammelide




Ammeline




Cyanuric acid

**Figure 1:** Structures of melamine (2,4,6-triamino-1,3,5-triazine), cyanuric acid (2,4,6-trihydroxy-1,3,5-triazine), ammeline (4,6-diamino-2-hydroxy-1,3,5-triazine) and ammelide (6-amino-2,4-dihydroxy-l,3,5-triazine)

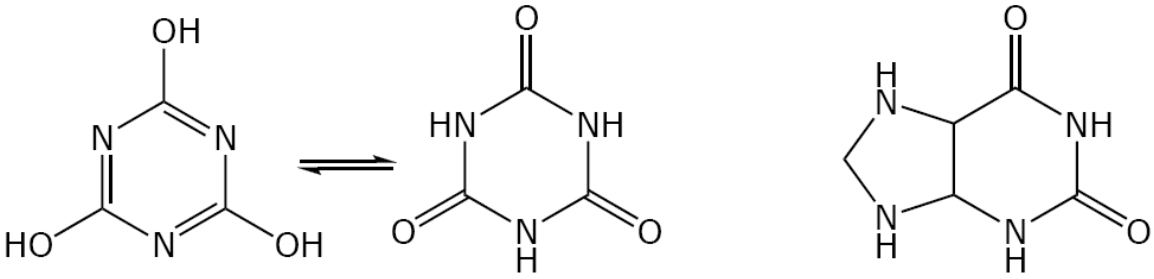
Ammeline (CAS No. 645-92-1), ammelide (CAS No. 645-93-2) and cyanuric acid may be produced by microbial degradation of melamine by sequential hydrolysis to replace one, two and then three amino groups, respectively. Cyanuric acid occurs also as a dissociation product of dichloroisocyanurates used in disinfection of water (e.g. swimming pools) and food contact materials.

[15] A number of other triazine compounds are used in some countries as herbicides or dyes. These compounds are not considered relevant to this opinion because they do not have three amine groups present in the 2, 4 & 6 positions of the triazine ring and are therefore not precursors of melamine.

The occurrence of melamine, cyanuric acid, ammelide and ammeline from the authorised uses of melamine and cyanuric acid is sometimes referred to as background levels.

Previous investigations indicated that the toxicity of melamine and cyanuric acid is comparatively low, with lethal dose ($LD_{50}$) values in excess of 1 g compound / kg body weight (b.w.) (NTP, 1983; Hammond et al., 1986; Hodge et al., 1965). However, animal studies also indicated that melamine can cause crystalluria in rodents (mice, rats) and in dogs and cats and fatal uremia following crystalluria in sheep (Osborne et al., 2009).

Recently, there have been a number of incidents of adulteration or contamination of feed and food with melamine. In 2007 illness and death in pet cats and dogs in the United States of America (USA) were associated with pet food manufactured with wheat gluten or rice protein concentrate imported from China, which were found to contain melamine and cyanuric acid (the so-called scrap melamine). Crystals containing melamine and cyanuric acid were found to be present in the kidneys of animals that died. In late 2008, approximately 300,000 infants in China were affected by infant formula containing melamine, including six deaths confirmed to be due to melamine (WHO, 2009a). Many of the affected infants had stones or calculi in the kidney, ureter or bladder, composed of melamine and uric acid (see chemical structure in Figure 2), the latter being naturally present in urine. The difference between the pets and human infants in the composition of their urinary stones appears to be due to the source of melamine to which they were exposed. The melamine added to the infant formula contained very low levels of cyanuric acid whereas the pet food contained a crude mixture of melamine and cyanuric acid. Melamine has also been found in soybean meal intended for feed, and in ammonium bicarbonate used in the food industry, although there are no known incidents of animal or human ill health associated with these.




Cyanuric acid and tautomeric form Uric acid

**Figure 2:** Structures of cyanuric acid tautomers and uric acid

Recent findings suggested that combined exposure to melamine and cyanuric acid may result in the formation of a 1:1 molecular complex denoted MCA (melamine–cyanuric acid) complex. The crystal structure of the MCA complex in the presence of Hydrochloric acid (HCl) was described in detail by Wang et al. (1990), indicating various hydrogen bonds and a laminar configuration of the edged crystals. Although characterisation of the parameters that drive MCA complex formation is incomplete, the solubility of the MCA complex is estimated to be approximately 1000-fold less than that of the individual compounds. Such MCA complexes have been reported in uroliths isolated from the urinary tract of pet animals with cystitis. In addition, there is direct evidence that combined exposure to both compounds is more nephrotoxic than exposure to melamine or cyanuric acid alone. Pathological examination of affected animals demonstrated the presence of crystals in the urinary bladder and upper urinary tract in dogs. The nature of the crystals was characterized by direct examination (chemical and spectral analysis), and by the characteristic histopathological effects of the crystals on the renal structures (Dobson et al., 2008; Xie et al., 2009). Taken together, these data suggest that the renal insufficiency observed in a large number of dogs is the consequence of mechanic tissue damage by crystals, followed by blockage of the kidney tubules and urinary reflux progressing

into severe renal damage (Dobson et al., 2008). This pathogenesis resembles many features of the acute urate nephropathies in human infants.

In the incident related to infant food in China in 2008, the adulteration was with relatively pure melamine, with little residual amounts of cyanuric acid. In a recent case report, Fourier transform infrared spectroscopy and scanning electron microscopy of the calculi obtained from the urinary bladder of an 11 month old girl indicated indeed only the presence of melamine and uric acid, suggesting complex formation and subsequent crystallisation in the absence of cyanuric acid (Grases et al., 2009). The nature of these crystals makes it likely that these are a cause of primary renal damage in infants with an acidic urinary pH and a high concentration of uric acid in the urine. Taken together, these findings suggest that at acidic pH, melamine is able to form complexes with endogenous uric acid excreted by humans into urine, particularly during infancy.

### 1.1. Previous assessments

The Scientific Committee for Food (SCF) established a tolerable daily intake (TDI) of 0.5 mg/kg (b.w.) per day for melamine evaluated for use in food contact materials, but no details were given regarding the basis for the derivation (EC, 1986). Following the reports of illness in pets in 2007, the United States Food and Drug Administration (US FDA) published an interim risk assessment on risks to human health associated with consumption of products from animals inadvertently given feed adulterated with melamine and its analogues (US FDA, 2007). The US FDA set a TDI of 0.63 mg/kg, based on the no-observed adverse effect level (NOAEL) of 63 mg/kg b.w. per day for bladder stones in the second NTP 13-week feeding study (as cited in OECD, 2002), and an uncertainty factor of 100. The NOAEL of 63 mg/kg b.w. per day appears to have been derived from the dietary concentration of 750 mg/kg, assuming a daily consumption of 10 g dry laboratory chow by young rats (WHO, 1987) and the mean body weight of the rats at the start of the study. Also in 2007, The European Food Safety Authority (EFSA) provided an urgent provisional statement on the risks to animal and human health related to the presence of melamine and structurally related compounds in protein-rich ingredients used for food and feed (EFSA, 2007). EFSA noted that the TDI set by the US FDA was in line with that derived by the SCF. In the same statement, EFSA proposed a TDI of 1.5 mg/kg b.w. per day for sodium cyanurate, based on results of a 2-year feeding study in rats in which the NOAEL was 154 mg/kg b.w. per day, applying an uncertainty factor of 100 (EFSA, 2007).

Following the milk formula incident, EFSA received a further request from the European Commission for urgent advice, and issued a statement in September 2008 (EFSA, 2008a). EFSA estimated dietary exposure from consumption of composite foods in the European Union (EU) that could contain potentially adulterated milk powder from China and compared this with the TDI of 0.5 mg/kg b.w. per day set by the SCF.

In 2008, the FDA issued two updated risk assessments, both referring to the 2007 TDI, and the NOAEL for melamine of 63 mg/kg b.w. per day from which it was derived. The first update considered a TDI for combined exposure to melamine and analogues such as cyanuric acid. An additional uncertainty factor of 10 was used to set a TDI 0.063 mg/kg for melamine and its analogues, allowing for the uncertainty associated with increased toxicity due to the combination (US FDA, 2008a). The US FDA also noted that this TDI did not apply to consumption of infant formula for a number of reasons including potential contamination with more than one melamine analogue, that formula could be the sole source of nutrition and that it could be consumed by infants and toddlers whose renal systems may not be fully developed. In the second update, exposure of infants was specifically considered, taking into account that combinations of melamine and cyanuric acid had not been detected in infant formula in the USA. An additional uncertainty factor of 10 was applied to the 2007 TDI, to set a TDI 0.063 mg/kg for exposure of infants to melamine alone in infant formula (US FDA, 2008b). The reason for the additional uncertainty factor in this instance was that infants "*may be more sensitive than adults to exposures because, for example, infant formula is the sole source of nutrition, exposure continues for up to 12 months and renal function may be immature compared to adults*".

A WHO expert meeting was held in December 2008 to evaluate the toxicological and health aspects of melamine and cyanuric acid (WHO, 2009a). The meeting concluded that the epidemiological data did not allow a detailed dose response assessment and that it was necessary to rely on the toxicological studies for risk assessment purposes. The WHO established a TDI of 0.2 mg/kg b.w. per day for melamine (i.e. 200 µg/kg) (WHO, 2009a). The WHO noted that the incidence of bladder stones in the male rats treated at the "NOAEL" of 63 mg/kg b.w. per day, whilst not statistically different from control, was part of a significant dose response relationship. Therefore as an alternative to the NOAEL approach the WHO conducted dose-response modelling in order to fully capture the general pattern of the dose response relationship and calculated a benchmark dose lower confidence limit for a 10 % increased incidence of bladder stone development ($BMDL_{10}$). The $BMDL_{10}$ was 415 mg/kg diet, which was converted to a dose of 35 mg/kg b.w. per day applying the standard dietary conversion factor of 0.10 for young rats (i.e. assuming 10 g food consumed per day) taking into account an "a*dditional feed reduction adjustment factor of 14 % as observed in the second subchronic study*". A total uncertainty factor of 200 was applied, comprising a factor of 100 for intra- and inter-species variability and an extra uncertainty factor of 2 to "*fully account for the potential increased senstivity of infants and for data uncertainties*". No explanation was provided for the value of 2 selected for this additional uncertainty factor. The resultant TDI for melamine was rounded to a single significant figure of 0.2 mg/kg b.w. per day and was considered applicable to the whole population, including infants. This assessment was specific to exposure to melamine alone. It was considered that combined exposure to melamine and cyanuric acid would be more toxic than separate exposures but that there were insufficient data on which to develop a hazard characterisation for such scenarios. Based on the TDI of 1.5 mg/kg b.w. per day for cyanuric acid, the WHO suggested that exposure to melamine analogues such as ammeline and ammelide would be no more toxic than exposure to melamine. However, the available data were not adequate to allow the calculation of a health-based guidance value for simultaneous exposure to melamine and its analogues.

### 1.2. Physicochemical properties of melamine and analogues

Melamine (2,4,6-triamino -1,3,5-triazine) is a white powder as traded commercially with a high melting point of ca. 354°C. It is slightly soluble in cold water (ca. 3.1 g/L at 20°C and, as expected from its weakly basic character (Figure 1) it is more soluble at acidic pH. It is sparingly soluble in polar organic solvents (e.g. <1 g/L in 95 % ethanol and acetone) and essentially insoluble in non-polar solvents such as benzene, hexane and ether (OECD, 2002).

Cyanuric acid (2,4,6-trihydroxy-1,3,5-triazine) is a crystalline powder with a high melting point of about 360°C. It is sparingly soluble in cold water (ca. 2g/L) and rather insoluble in both polar and non-polar organic solvents. As expected from its acidic character (Figure 1) it is more soluble at alkaline pH.

Ammeline (4,6-diamino-2-hydroxy-1,3,5-triazine) is a white powder which decomposes before melting. It has weakly acidic properties and it is soluble in both aqueous alkalies and mineral acids.

Ammelide (6-amino-2,4-dihydroxy-l,3,5-triazine) is a white powder that is virtually insoluble in water. It decomposes at 170$^{o}$C to form carbon dioxide and ammonia (Bann and Miller, 1958).

### 1.3. Physico-chemical properties of melamine and of the melamine: cyanuric acid complex

Melamine can form co-precipitates with uric acid (occurring naturally in urine) or cyanuric acid (excreted into urine when co-exposure occurs). These complexes are very stable and can be hydrolysed at very low or very high pH.

Tolleson et al. (2009) demonstrated that at neutral pH the affinity of melamine for uric acid was 29-fold less than the affinity of melamine for cyanurate. However, at pH 4, a 6.4-fold tighter binding was found for melamine-urate in comparison with the affinity at pH 7, based on the increase in the dissociation constant $K_d$ with increasing pH (140 , 204, 490 and 900 µmol/L at pH 4.0, 5.0, 6.0 and

7.0, respectively). So, in contrast to the melamine-cyanurate complex, the stability of melamine-urate complexes decreases with increasing pH. Grases et al. (2009) stated that in their "in vitro" study melamine and uric acid formed an insoluble compound at urinary pH <5.0. Uric acid and melamine mixed in aqueous media at pH values > 5.5 formed no crystals. Since melamine is soluble in water, and uric acid is in its anionic form at pH values >5.5, formation of insoluble solid can only take place at acidic pH values.

The stability of melamine-cyanurate co-crystals under various pH conditions has been discussed by Tolleson (2008). *"Melamine and cyanuric acid are known to form a network of well-ordered intermolecular hydrogen bonds that self-assemble spontaneously. The keto-form of cyanuric acid in equilibrium with the enol-form is the form involved in the hydrogen bonded network with melamine. Solvent pH affects the extent to which both melamine and cyanuric acid exist in their un-ionized forms that are available for hydrogen bonding. Although cyanuric acid is triprotic and melamine is tribasic, their first ionizations (pKa 4.74 and 5.34, respectively) are physiologically relevant within pH 5.0 – 7.3 found in the kidney. Below pH 6 melamine is converted from the uncharged free amine form to the melamine ammonium cation, destabilizing hydrogen bonding with the keto-form of cyanuric acid. Similarly, cyanuric acid dissociates above pH 4 to form its conjugate base, also destabilizing the melamine – cyanuric acid complex. Optimal concentrations of free acid and free base forms available to form hydrogen bonds can be calculated from the average of the two pKa values, e.g. pH 5.04. At conditions of constant ionic strength similar to plasma (I=0.15) and over a pH range spanning that of the nephron, a solubility minimum was revealed for melamine and cyanuric acid) close to pH 5."*

In additional correspondence it was explained that the solubility curves in the figure from Tolleson et al. (2009) reflect the solubility of the melamine-cyanurate complex at different pH. While at low ionic strength the complex of the stability is reduced at pH< 5, the stability is hardly affected at higher pH. However at higher ionic strength (I = 1; reflective of the ionic strength at some parts in Henle's loop) also at pH > 6.5 a decrease in complex stability can be demonstrated (data not shown)[16]. Dominguez-Estevez et al. (2010) investigated the solubility of melamine alone and in combination with cyanuric acid in urine of healthy human adults and of rats. A strong pH-dependency was observed with the lowest solubility found at pH 5-5.5. At pH 5.5, the solubility of melamine in rat urine was 6.9-fold higher than that in human urine.

## 1.4. Methods of analysis

### 1.4.1. Analytical methods in food and feed materials

Several analytical methods for the determination of melamine (with and without analogues) in various matrices have been developed and published. Following the incident of adulterated pet food, methods were developed for composite foods and for individual ingredients too. Similarly, following the incident of adulterated milk, methods were developed for milk, milk powder, composite products containing milk, eggs, and then for foods generally. As a result, reliable and sensitive analytical methods are available for most food types, including foods high in protein, fat and carbohydrates. The main focus of method development and analysis has been on melamine and (to a lesser extent) cyanuric acid, since the initial incident with pet food involved co-adulteration with these two substances.

Liquid extraction of the food is the first step and the extract is usually treated by a technique such as solid phase extraction on a disposable cartridge to both clean-up the sample and to allow concentration to aid sensitivity. The analytical method used most commonly and with the best detection capability and selectivity (giving confidence in correct identification and quantitation) is LC-MS/MS (liquid chromatography coupled to mass spectrometry). GC-MS (gas chromatography coupled to mass

[16] Personal communication between Dr. WH Tolleson (United States Food and Drug Administration – US FDA) and Dr. W. Mennes (The Dutch National Institute for Public Health and the Environment - RIVM) made available to EFSA.

spectrometry) is also sometimes used and it can give reliable results, but melamine and analogues require derivatisation to make them volatile. LC-UV (liquid chromatography with or without diode array detection (DAD)) is also used but has lower sensitivity and identification power and so is best suited for low-cost screening analysis and for the analysis of simple samples at such as the food simulants used in migration testing. With LC-MS operated in the positive ionisation mode for melamine, the analogues ammeline and ammelide can be tested for in the same analytical run. Unfortunately, cyanuric acid has different physicochemical proprieties and it needs ionisation in the negative mode for best sensitivity. Cyanuric acid can also suffer from interferences from coextractives from some food types if analysis is attempted simultaneously with the three other substances and using an MS polarity switch to allow the detection of positively-charged (melamine, ammeline and ammelide) and negatively-charged (cyanuric acid) ions Consequently cyanuric acid is often tested for in a separate analysis. Isotope-labelled ($^{13}C_3$-) melamine is widely used as an internal standard, which helps the reliability of measurements. Measuring down to ca. 50 µg/kg for each of the 4 substances of interest is possible now for most sample types of interest. A review of the analytical methods used for melamine and related compounds has been published very recently (Tittlemier, 2010).

#### 1.4.1.1. The reliability of analytical results and occurrence (concentration) data

The Analytical Quality Control steps necessary to ensure reliable measurements were summarised in the WHO review and in the recent review of methods for analysis of melamine and related compounds (WHO, 2009a, Tittlemier, 2010). In the evaluation of occurrence data, provided that a sufficient number of data points are available, then the main question is the accuracy of the data - meaning freedom from bias - rather than the precision of the data - normally summarised as the measurement uncertainty. For the analysis of melamine and analogues four main sources of potential bias can be identified and discussed below: are incomplete extraction, interferences, matrix supression, and cross reactivity.

#### 1.4.1.2. Extraction procedure

All proper analytical methods should include a validation step to determine the analytical recovery. One area of early concern was the limited solubility of the melamine: cyanuric acid complex. Many analytical methods use water, acetonitrile or alcohol, or mixtures thereof, as the extraction solvent. As stated above, the water solubility of melamine and cyanuric acid alone is in the range 2-3 g/L whereas the solubility of the complex is much lower at about 0.01 g/L (Tittlemier, 2010). An extraction procedure that is adequate for melamine or cyanuric acid alone may not be strong enough to extract the complex. Considering the dissociation constant for the complex and also taking account of the ratio of sample mass to extraction solvent volume used typically, this problem should not arise at the 2.5 mg/kg action level in foods nor at the diluted concentrations used for analytical (calibration) standards. If significant co-contamination of a food is suspected (as was found to be the case in the pet food incident) then procedures are available to improve the solubility e.g. by adding acetonitrile as a co-solvent and/or breaking the complex by using some dimethylamine in the extraction solvent. If this aspect is not under control then analytical results could have a negative bias, meaning that they are too low. This will affect only samples containing very high levels of both melamine and cyanuric acid which was not the case for the milk incident.

#### 1.4.1.3. Interferences

If a rather non-specific analytical method is used such as LC-UV, and especially with earlier reverse-phase LC columns that did not have good retention or separating power for polar molecules, there is the potential for mis-identification of melamine giving a positive bias, i.e. results that are too high. For migration studies conducted in the past, testing against a specific migration limit (SML) of 30 mg/kg meant that LC-UV was invariably the method of choice and since the SML was rarely if ever exceeded then the results were never followed up using LC-MS. The possibility of ammeline or ammelide migration was not recognised and they (but not cyanuric acid) may have co-eluted using the

LC columns of the day. The net result is that results of tests conducted before ca. 2005 (melamine in pet food incident) are more likely to be biased high rather than low.

#### 1.4.1.4. Matrix suppression

A similar problem but with the opposite result can occur even if LC-MS is used. The problem is again one of co-elution (particularly with old column chemistries that have poor separating power) but, unlike with a UV detector where an artificially high detector response will be obtained, in MS a coelutant can interfere with the ionisation process giving rise to matrix suppression and an underestimation of the true concentration. This is easily avoided by the use of matrix-matched calibration standards or the analysis of spiked controls, which should be normal practice.

#### 1.4.1.5. Cross-reactivity

A small number of ELISA (enzyme-linked immunosorbent assay) kits are available commercially for the analysis of melamine. These kits have been validated for only a few sample types of food and feed. The two main pitfalls of ELISA are matrix interference (giving low results) and cross-reactivity giving high results. Matrix interference can be guarded against by over-spiking the samples or more completely by the use of the method of standard additions. Cross-reactivity (against either extraneous or intrinsic food chemicals) cannot be checked so easily and so validation for example by cross-comparison with alternative methods such as LC-MS is needed.

#### 1.4.1.6. Conclusion on the reliability of analytical results

Reliable extraction and sample clean-up techniques are available for most food types including foods high in protein, fat and carbohydrates. The most sensitive and selective analytical method to measure melamine and its structural analogues is LC-MS/MS (liquid chromatography coupled to tandem mass spectrometry) although other reliable methods are available too. For example, in the recent food analysis performance assessment scheme (FAPAS) round on melamine in chocolate, 83 test materials were sent to 37 countries and 72 participants returned results within the timeframe allowed. The assigned concentration of melamine was 5.69 mg/kg. The boundaries for satisfactory performance were set at 4.29 to 7.09 mg/kg and 49 participants (68 %) achieved satisfactory scores. 65 of the 72 (90 %) participants returned results within +/- 50 % of the assigned value (i.e. in the range 2.35 to 8.04 mg/kg). The majority of participants used LC-MS, the next most common approach was using LC-UV, then GC, and one lab reported using ELISA. All results were distributed symmetrically around the central tendency and there was no evidence of bias overall (FAPAS, 2010).

### 1.4.2. Analytical methods used in clinical samples

For humans and pet animals, non-invasive ultrasound examination of the urinary system has been used to image precipitates of melamine-complexes. Uroliths collected from pet dogs have also been analysed using scanning electron microscopy and infrared spectroscopy (FTIR), solid state nuclear magnetic resonance (NMR) spectroscopy, X-ray diffraction and energy disperse spectroscopy to study their chemical composition (Osborne et al., 2009).

## 2. Legislation

The main legislation relevant to potential human exposure to melamine and its analogues are identified as follows: (i) Specific migration limit for melamine in food contact materials; (ii) emergency measures on maximum melamine contamination in food and feed from China; (iii) legislation for melamine in other food and feed; (iv) legislation for melamine precursors; (v) legislation for melamine precursors in flame retardants (vi) sodium dichloroisocyanurate used as a biocide. Melamine is used as a feed additive for animals in some countries but this use is not permitted in the EU and so this

application is not described further. The permitted applications and the relevant EU regulations are summarised below.

## 2.1. Melamine

### 2.1.1. Specific migration limit for food contact materials

In the European Union, melamine is approved for use as a monomer and as an additive in plastics and it has a specific migration limit (SML) of 30 mg/kg food (Commission Directive 2002/72/EC of 6 August 2002 relating to plastic materials and articles intended to come into contact with foodstuffs[17)]. This was derived from the TDI of 0.5 mg/kg b.w. per day and the default exposure scenario that is used for plastic food contact materials (FCMs) of an intake of 1 kg packaged food per day by a 60 kg b.w. adult. There are no specific EU regulations on melamine used in non-plastics (e.g. paper and board, can coatings) but the SML of 30 mg/kg food is often taken as a presumptive standard.

### 2.1.2. Maximum contamination in food and feed originating from China

In 2008, a Community emergency measure was introduced following findings of high levels of melamine in infant milk and other milk products in China. The import into the EU of (raw) milk and (raw) milk-based products originating from China (defined as containing more than 50 % milk) was already prohibited by Commission Decision 2004/438/EC of 29 April 2004 laying down animal and public health and veterinary certifications conditions for introduction in the Community of heat-treated milk, milk-based products and raw milk intended for human consumption[18].The import into the EU of heat treated milk and milk-based products originating from China would be allowed from establishments listed in Annex to Commission Decision 97/252/EC of 25 March 1997 drawing up provisional lists of third country establishments from which the Member states authorize imports of milk and milk products for human consumption[19]. However, as no establishment is listed for China in Annex to Commission Decision 97/252/EC, the import of heat treated milk and milk based products is not allowed. Furthermore Article 29 (1) of Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products[20] provides that the import of animals and animal products from a third country is subject to the submission by the third country and approval by the Commission of a residue monitoring plan. As no residue monitoring plan from China for milk has been approved by Commission Decision 2004/432/EC of 29 April 2004 on the approval of residue monitoring plans submitted by third countries in accordance with Council Directive 96/23/EC[21], the import into the EU of milk and milk products from China is also, by virtue of this legislation, not allowed.

Commission Decision 2008/798/EC of 14 October 2008 imposing special conditions governing the import of products containing milk or milk products originating in or consigned from China (repealing Commission Decision 2008/757/EC) [22] prohibited the importation from China of products containing milk or milk products, soya or soya products intended for the particular nutritional use of infants and young children. It also required Member States to check all consignments from China of feed and food containing milk or milk products, soya or soya products. An action level of 2.5 mg/kg was set by the Commission to distinguish between the unavoidable background presence of melamine and possible adulteration. Furthermore, an increased control of food and feed containing high protein

[17] OJ L 220, 15.08.2002, p.18-58.
[18] OJ L 154, 30.4.2004, p. 72, corrected by OJ L 92, 12.4.2005, p.47-50.
[19] OJ L 101, 18.4.1997, p. 46-104.
[20] OJ L 125, 23.5.1996, p. 10-32.
[21] OJ L 154, 30.4.2004, p. 44-50, corrected by OJ L 189, 27.5.2004, p. 33-39.
[22] OJ L 273, 15.10.2008, p.18-20.

levels were recommended at this action level. Commission Regulation (EC) No 1135/2009 of 25 November 2009 imposing special conditions governing the import of certain products originating in or consigned from China, has now repealed Decision 2008/798/EC[23]. Based on a significant decrease in Rapid Alert System for Food and Feed (RASFF) notifications the intensity of mandatory physical checks was reduced although the action limit of 2.5 mg/kg was maintained.

### 2.1.3. Legislation for melamine in other food and feed

There is no specific legislation for melamine in other foods and feed but the action level of 2.5 mg/kg has been applied by member states to distinguish between unavoidable background presence of melamine and adulteration in cases where no migration from food packages could be demonstrated.

### 2.1.4. Legislation for melamine precursors

Melamine may arise in foods as a breakdown product of the active substance cyromazine which is authorised for use in Europe both as a pesticide (insecticide and acaricide) and as a veterinary medicinal product (ectoparasiticide). Cyromazine can be used as a systemic insect growth regulator to control leaf miners in vegetables such as celery, lettuce, melons, mushrooms, lettuce and potatoes. In plants, cyromazine is rapidly metabolised including to melamine as a minor metabolite. The general principals of establishing Maximum Residue Limits (MRLs) for pesticides are laid down in Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. The current MRLs for cyromazine are established in Regulation 839/2008[24]. The majority of these MRLs are set at the limit of quantification (0.02 – 0.05 mg/kg) reflecting that the use of cyromazine containing plant protection products is not authorised for these commodities. For the approved uses, MRLs for cyromazine are in the range of 0.3 mg/kg (e.g. cucurbits) up to 15-20 mg/kg (e.g. leafy vegetables and beet leaves respectively). Melamine is not included in the enforcement residue definition, but it is included in the residue definition for risk assessment of the MRL and the estimation of the residues of melamine from cyromazine sources is dealt with in the subsequent sections of the opinion.

Cyromazine is used as a veterinary drug in the treatment of poultry feed to control diptera larvae in chicken manure, and is also administered as a pour-on to prevent blow-fly strikes on sheep. In animals, cyromazine is metabolised to melamine. There are 4 MRLs relating to the veterinary use of cyromazine: 0.3 mg/kg for ovine fat, liver, kidney and muscle (Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin[25]). Cyromazine is not permitted for use on lactating animals producing milk for human consumption. Melamine, as the major metabolite of cyromazine, is not included in the residue definition of these MRLs and the estimation of the residues of melamine from cyromazine sources is dealt with in the subsequent sections of the opinion.

### 2.1.5. Legislation for melamine precursors in flame retardants

Melamine and its salts with acids such as cyanuric acid, boric acid and phosphoric acids are available commercially as fire retardants, also known as flame retardants. Apart from the normal requirements for the notification of substances that are new to the European market under REACH regulation 1907/2006, no melamine-related flame retardant are subject to an authorisation procedure. Similarly, although several flame retardants have been listed on the priority lists requiring a comprehensive risk

[23] OJ L 311, 26.11.2009, p. 3–5.
[24] OJ L 234, 30.8.2008, p. 1–216.
[25] OJ L 015 , 20.01.2010, p. 1-72.

assessment under the Council Regulation (EEC) N0 793/93 of 23 March 1999 on the evaluation and control of risks of existing substances[26], melamine-related substances are not amongst them.

## 2.2. Cyanuric acid

### 2.2.1. Cyanuric acid for biocide use

Biocides are regulated at EU level by Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market[27]. Pursuant Directive 98/8/EC, Member States may only authorise the placing on the market of biocidal products containing active substances included in Annex I, IA or IB to that Directive. Cyanuric acid is not listed in these annexes and is therefore not allowed to be used as a biocide in the EU. Biocidal products containing an active substance not listed in the Annex I, IA or IB to the Directive 98/8/EC, which were on the market on 14 May 2000, are called "existing active substances"). Cyanuric acid is an "existing active substance".

All existing active substances are subject to a 10-year review programme and the biocidal products containing an active substance subject to a review under the review programme may remain on the market until a decision on inclusion or non-inclusion in Annex I, IA or IB to Directive has been taken.

However, cyanuric acid is an existing active substance in respect of which no notification has been accepted or no Member State has indicated an interest to support inclusion in Annex I, IA or IB to Directive 98/8/EC. Therefore cyanuric acid was not included in the review programme. For biocidal products containing an active substance not included in the review programme (and not included in Annex I, IA or IB), Member States had to cancel existing authorisations or registrations for such biocidal products and to ensure that such biocidal products are no longer placed on their market as from 1 September 2006. Consequently cyanuric acid has been used as biocidal product in the EU in the past but is no longer authorised to be used since 1 September 2006.

Sodium dichloroisocyanurate is an organic chlorine donor that has been used as an alternative disinfectant to other chlorine sources such as sodium hypochlorite. Applications of sodium dichloroisocyanurate as a disinfectant in food, feed and drinks include drinking water, swimming pool water and topical use in animal husbandry, and products such as equipment, containers, consumption utensils, surfaces or pipework associated with the production, transport, storage or consumption (WHO, 2007, 2009b). Sodium dichloroisocyanurate dihydrate is not listed in these Annex I, IA or IB to that Directive Pursuant Directive 98/8/EC annexes.

Sodium dichloroisocyanurate dihydrate is an existing active substance in respect of which initially a notification was received under the review programme for following product types (Commission Regulation (EC) No 1451/2007):

- disinfectants and general biocidal products;
- human hygiene biocidal products (type 1);
- private area and public health area disinfectants and other biocidal products (type 2);
- veterinary hygiene biocidal products (type 3);
- food and feed area disinfectants (type 4);
- drinking water disinfectants (type 5);
- preservatives;
- in-can preservatives (type 6):
- fibre, leather, rubber and polymerised materials preservatives (type 9);
- preservatives for liquid-cooling and processing systems (type 11);

[26] OJ L 84, 5.4.1993, p. 1.
[27] OJ L 325, 11.12.2007, p. 1.

- slimicides (type 12).

However, in the meantime no interest was shown and no complete dossier was received for:
- product type 1, human hygiene biocidal products (Commission Decision 2008/809/EC);
- product type 6, in-can preservatives (Commission Decision 2008/809/EC);
- product type 9, fibre, leather, rubber and polymerised materials preservatives (Commission Decision 2010/72/EC).

Consequently, sodium dichloroisocyanurate dihydrate was no longer allowed to be placed on the market as from 24 October 2009 for type 1 and 6 uses and shall no longer be placed on the market for type 9 uses with effect from 2011.

Pending the finalisation of the review programme, the placing of the market for the other product type uses (2, 3, 4, 5, 11 and 12) is allowed.

### 2.2.2. Cyanuric acid in animal feed

Urea-based feed additives such as biuret can contain impurities such as melamine and cyanuric acid. Biuret (97 % technically pure) was authorised for use in feed in the EU since 1982 as a non-protein nitrogenous compound by Council Directive 1982/471/EEC of 30 June 1982 concerning certain products used in animal nutrition[28] . By Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition[29] biuret was classified from 18 October 2004 as feed additive falling within the category "nutritional additives", functional group "urea and its derivatives". Biuret has been included in the Community Register of Feed Additives[30].

In the USA, cyanuric acid is regulated as a component (up to 30 %) of feed-grade biuret[31].

## 3. Uses

The main uses of melamine and precursors are summarised below and include food contact materials and articles such as melamine-formaldehyde ('melaware'), can coatings, paper and board, adhesives and a pesticide used in plant protection and veterinary applications.

## 3.1. Melamine

### 3.1.1. Food contact materials and articles

Melamine is reacted with other starting substances, mainly formaldehyde (Figure 3) and urea, into a variety of high molecular weight resins and plastics that are intended to come into contact with foods. These include durable articles such as cups, plates and kitchen utensils, protective coatings used on the inside of metal food cans, and melamine resins used in paper-making and adhesives. EU regulations on plastics also allow the use of melamine as an additive as well as a starting substance. The distinction is that a starting substance is used-up by chemical reaction and so any residue of that chemical that remains in the finished material or article is usually unintended. In contrast, an additive is intended to be present in the polymer or in the finished material or article in sufficient concentration to achieve a technical effect. Only one application as an additive in food packaging was identified;

[28] OJ L 213, 21.7.1982, p. 8.
[29] OJ L 268, 18.10.2003, p. 29.
[30] See http://ec.europa.eu/food/food/animalnutrition/feedadditives/comm_register_feed_additives_1831-03.pdf
[31] See http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=573.220

this was using melamine as a thin layer in laminates in order to provide gas barrier properties. All other applications known are for use as a starting substance.

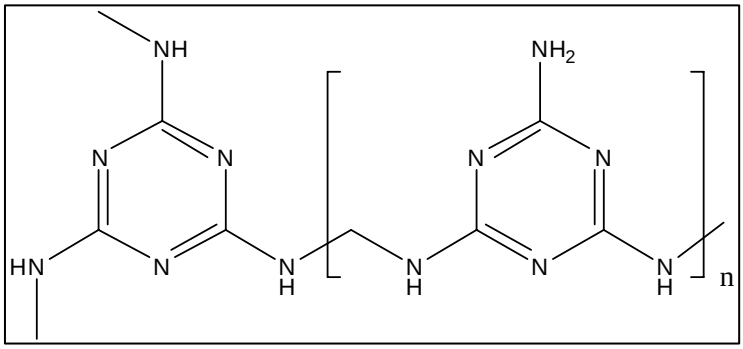



**Figure 3:** Melamine formaldehyde polymer

3.1.1.1. Melamine-formaldehyde (known colloquially as 'melaware') articles:

Melamine is reacted with formaldehyde (Figure 3) to produce a thermoset plastic called 'melamine' or 'melaware'. Typical food contact applications of melaware are children's mugs, bowls, cups, plates and other kitchen utensils.

3.1.1.2. Melamine – based can coatings

Melamine and derivatives such as methylolated melamine are also used as starting substances for coatings used inside food cans. The can coatings are stoved (cured) at high temperatures to give extensive cross-linking. The coatings are very thin, at typically 5 to 10 µm, so that the potential for migration of residual (unreacted) melamine is expected to be low. On the other hand, the contact conditions for canned food and beverages can be very demanding, including sterilisation or pasteurisation of the foodstuff in the can at high temperatures and then subsequent long-term storage for up to several years. Consequently, the stability of the coatings themselves, and of any low molecular weight melamine derivatives used as precursors, needs to be considered too.

3.1.1.3. Paper and board coated with melamine based polymers

Wet-strength agents used in paper-making may contain melamine polymers, with possibly the presence of residual melamine monomer (FSA, 2006).

3.1.1.4. Adhesives from melamine-formaldehyde resins

Adhesives used to make food packaging may contain modified melamine resins. It may be expected that because of the low amounts used, the fact that melamine would be used as a reactive component (and so be used up) and because of the presence of barrier layers (adhesives not used in direct contact with the food) then migration levels could be expected to be low.

3.1.1.5. Melamine gas barrier coatings

Proprietary technology exists to vacuum deposit melamine and other symmetrical organic molecules onto flexible packaging materials to create a thin layer with high gas barrier properties (DSM, 2009).

3.1.1.6. Use of melamine precursors

Cyromazine is used both as a plant protection product (insecticide and acaricide) and a veterinary medicinal product (ectoparasiticide for special use in sheep). As a systemic insect growth regulator, it is usually used as a foliar spray to control leaf miners in vegetables such as celery, lettuce, melons, mushrooms, lettuce and potatoes. In plants (as well as in animals), cyromazine is rapidly metabolised including to melamine as a minor metabolite (JMPR, 2007).

Cyromazine is used in poultry farms. It is applied orally to control diptera larvae in chicken manure. In sheep it is administered as a pour-on to prevent blow-fly strikes. Usage is not permitted on lactating animals producing milk for human consumption.

3.1.1.7. Use of melamine and its salts as flame retardants

Melamine and its salts with acids such as cyanuric acid, boric acid and phosphoric acids are available commercially as fire retardants, also know as flame retardants. They are sold for applications including foams, textiles, plastics and coatings, used in products such as electrical appliances, furnishings, intumescent paints, fire doors and the like. Melamine and its salts are not expected to be accumulative and indeed they are some of the substitutes that are finding favour as alternatives to halogenated flame retardants and other chemical types that are both hazardous and persistent. So exposure via food possibly containing melamine from environmental contamination is not considered further. Some types of flame retardant can be lost through abrasion, leaching or volatilisation. While these processes may be very slow, an article's service life can be very long, and so these losses may be significant (Fisk et al., 2003). Considering exposure via the inhalation route, melamine itself has a high melting point of ca. 354°C and an insignificant vapour pressure can be calculated for ambient temperatures (Hirt et al., 2003). The melamine salts used as fire retardants can be expected to have similarly low vapour pressures. Consequently, outside of the fire scenario, human exposure through inhalation of vapours is considered to be insignificant. Inhalation via dust and particulates is an issue for occupational exposure for workers handling the powdered melamine and salts. It is considered unlikely that there is significant inhalation exposure of the general population via dust generated by abrasion of materials containing fire retardants, so this is not considered further here.

3.1.1.8. Melamine in animal feed

Melamine can be present as an impurity in urea-based commercial feed additives used in ruminants.

### 3.1.2. Cyanuric acid

3.1.2.1. Cyanuric acid in animal feed

Cyanuric acid can be present as an impurity in urea-based commercial feed additives such as biuret, authorised in the EU for use as feed additive as a 97 % pure formula. In contrast, in the USA, up to 30 % cyanuric acid is authorised in biuret. Cyanuric acid is not added intentionally to feed materials, but was found as a co-contaminant with melamine in adulterated pet food (Puschner et al., 2007).

3.1.2.2. Cyanuric acid precursors resulting from biocide use

Sodium dichloroisocyanurate is used as a source of active chlorine as a disinfection agent. It is available to consumers as tablets for sterilisation of baby bottles and drinking water (e.g. when hiking) and it is also used to treat the water used in swimming pools. Other applications include the cleansing of wounds and as a teat dip or spray in milking cows.

When added to water dichloroisocyanurate disassociates according to a complex series of equilibria involving six chlorinated and four non-chlorinated isocyanurates. As the active chlorine in solution is progressively consumed the equilibria shift to yield, ultimately, the non-chlorinated cyanuric acid (WHO, 2007; 2009b). For treating drinking water and swimming pool water, the amount of sodium

dichloroisocyanurate needed to be added will depend on the chlorine demand of the water and the desired concentration of residual (active) chlorine. The WHO calculated that at very high chlorine doses (up to 10 mg/L) the resulting concentration of (sodium) cyanurate would be up to 11 mg/L (WHO, 2007). Other potential sources of exposure to cyanuric acid resulting from the disinfectant use of dichloroisocyanurate would be minor. When used to sterilise baby bottles and other containers according to the instructions, the dilute solution will first be emptied out and then the bottle further rinsed out using potable water.

## 4. Occurrence

### 4.1. Data collection summary

Following the melamine incident of 2008, data on the occurrence of this contaminant have been collected by the European Countries and provided to the European Commission. Sampling was mainly targeted to particular food categories or geographical origins, including suspicious products as required by the legislation.

A large number of food and feed samples were analysed by the industry and have been provided to EFSA through industry associations. These included ingredients as well as products. Packaging materials have been tested with both simulants and real food products.

### 4.2. Data from European countries

The data submissions received from European Countries include only data on melamine and represent both the authorised use of melamine as a food contact material and possible misuse or adulteration.

A summary of the data submissions from European countries is reported in Table 1.

**Table 1:** Summary of the melamine data collection on food and feed from EU Countries during 2009. Number of samples, percentage of non-detected and limit of quantification are reported for each country submitting data.

| Country | Food | | | Feed | | |
|---|---|---|---|---|---|---|
| | N | % not detected | LOQ mg/kg | N | % not detected | LOQ mg/kg |
| Austria | 75 | 19 % | 0.10-0.50 | | | |
| Bulgaria | 7 | 100 % | 2.50 | | | |
| Cyprus | 5 | 100 % | 0.20 | | | |
| Czech Republic | 90 | 99 % | 0.05-2.50 | 4 | 100 % | 2.50 |
| Estonia | 5 | 80 % | 0.10 | | | |
| Finland | 79 | 92 % | 0.05-0.10 | 1 | 100 % | 0.10 |
| France | 128 | 99 % | 2.50 | 27 | 93 % | 2.50 |
| Germany | 136 | 4 % | 1.8 | 52 | 94 % | 2.5 |
| Hungary | 94 | 43 % | 0.1-2.5 | 2 | 100 % | 0.1-0.2 |
| Ireland | 48 | 4 % | 0.04 | 1 | 0 % | . |
| Italy | 339 | 95 % | 0.1-2.5 | 9 | 100 % | 2.38 |
| Latvia | 3 | 67 % | 0.1-2.5 | 1 | 100 % | 2.5 |
| Lithuania | 5 | 100 % | 0.1 | 1 | 100 % | 0.1 |
| Norway | 10 | 100 % | 0.1-3 | | | |
| Poland | 71 | 100 % | 2.5 | | | |
| Slovenia | 59 | 98 % | 2.5 | 37 | 100 % | 0.5-2.5 |
| Spain | 45 | 100 % | 2.5 | | | |
| Sweden | 40 | 100 % | 0.1-2.5 | 2 | 100 % | 2.5 |
| United Kingdom | 832 | 87 % | 0.05-3 | 29 | 100 % | 0.1-2.5 |
| Not classified | 2 | | | | | |
| *All* | *2073* | 79 % | *0.04-3* | *166* | 96 % | *0.1-2.5* |

N – number of samples; LOQ: limit of quantification.

The submissions range between a few samples and almost 900 samples, depending on the country. The percentage of non-detected varied among countries between 4.1 % and 100 %. This spread may be explained by a combined effect of the different numbers of samples and different levels of targeting. Overall, the European countries provided results from 166 feed samples and 2073 food samples, in total 2239 analyses. All the results were provided with the limit of quantification (LOQ).

### 4.3. Data from industry

The industry associations provided diverse data. The most extensive dataset was submitted by CIAA and included data from CIAA[32], CEPE[33] and Empac[34]. The sampling period spans between 2006 and 2009. Only 6 samples are reported from 2006, about 50 from 2007, more than 5000 from 2008 and more than 22000 from 2009. Ingredients and products from different world regions were tested for melamine and partly also for cyanuric acid. The distribution of analyses per geographical area and substance analysed is shown in Table 2.

[32] Confederation of the food and drink industries of the EU – www.ciaa.be
[33] European council of the Producers and Importers of Paints, Printing inks & Artists' colours – www.cepe.org
[34] European Metal Packaging – www.empac.eu

**Table 2:** Data submission from CIAA, CEPE and Empac, grouped by analysed substance and by geographical region.

| Geographical area | Number of Food samples | | Number of Feed samples | |
|---|---|---|---|---|
| | Melamine in food | Cyanuric acid in food | Melamine in feed | Cyanuric acid in feed |
| Africa | 1400 | 403 | 27 | 17 |
| Asia-Pacific | 7886 | 1395 | 149 | 52 |
| Central & South America | 2088 | 1180 | 172 | 97 |
| Europe | 5823 | 4371 | 110 | 105 |
| Middle East | 184 | 162 | 29 | 29 |
| North America | 557 | 425 | 17 | 6 |
| Not Classified | 600 | 123 | 6 | 4 |
| TOTAL | 18538 | 8059 | 510 | 310 |
| | 26597 | | 820 | |
| | 27417 | | | |

Both products for the final consumer and ingredients are represented in this dataset, as shown in Figure 4.

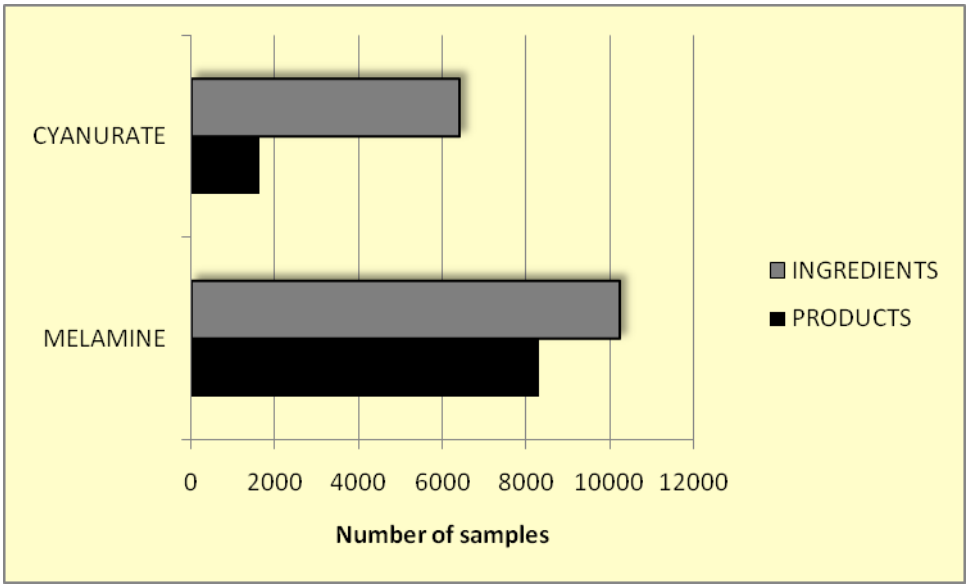



**Figure 4:** Data submissions on melamine and cyanuric acid occurrence in food from CIAA, CEPE and Empac, grouped by Products and Ingredients.

Ingredients represent foods and additives used by the industry for the manufacture of their products. Similar ingredients may be assumed, in the global market, to be used by other industries and even by the final consumer for home prepared food. For this reason the samples presented as products and those presented as ingredients were used together in the further calculations.

The limit of detection (LOD) was specified for less than 500 out of 27417 data points whereas numerical values were reported for all the other samples. A frequency analysis of these values reveals an unexpected high frequency of some figures. Table 4 shows the frequency of the fifteen first ranking values in the dataset.

**Table 3:** Number of occurrences of the fifteen most frequent values in the CIAA, CEPE and Empac Joint database.

| Reported value | Number of times the value occurs |
|---|---|
| 0.05 | 16746 |
| 0.3 | 2760 |
| 0.2 | 2152 |
| 0.1 | 1420 |
| 0.5 | 642 |
| 0.25 | 440 |
| 0.12 | 154 |
| 0.06 | 119 |
| 2.5 | 98 |
| 5 | 94 |
| 0.005 | 79 |
| 1 | 65 |
| 0.11 | 54 |
| 0.125 | 50 |
| 0.13 | 36 |

The high frequency of some figures suggests that values below the limit of detection have been reported in this database with the numerical value of the limit itself.

## 4.4. Occurrence of melamine in food and feed

### 4.4.1. Melamine occurrence data in food and feed provided by European countries

The overall 2239 values provided by European countries were grouped in categories according to the Concise Food Consumption database and descriptive statistics were calculated for each group. The "bounding" approach was applied for values reported below LOD or below LOQ in order to identify the possible range of the data. The lower bound (LB) is obtained by assigning a value of zero (minimum possible value) to all the samples reported as <LOD or <LOQ. The upper bound (UB) is obtained by assigning the value of LOD to values reported as <LOD and LOQ to values reported as <LOQ (maximum possible value). Because these were targeted samples focussing on products where adulteration was considered likely, they are not representative of background levels and are not used in the assessment of background dietary exposure.

**Table 4:** Occurrence values submitted by European countries for melamine (mg/kg) in food and feed grouped by sub-categories of the Concise Food Consumption groups. Upper and Lower bound values[*)] are reported.

| Concise categories | | | Lower bound (mg/kg) | | | | | Upper bound (mg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | <LOQ | min | Mean | Median | P95 | max | min | mean | median | P95 | max |
| 01A.Cereal-based mixed dishes | 146 | 73 % | 0 | 0.37 | 0 | 0.25 | 19.9 | 0.01 | 1.28 | 0.25 | 2.5 | 19.9 |
| 01B.Cereals & cereal products excl. cereal-based mixed dishes | 444 | 67 % | 0 | 1.6 | 0 | 10 | 57 | 0.01 | 2.52 | 1 | 10 | 57 |
| *01.Cereals & cereal products* | *590* | *68 %* | 0 | 1.3 | 0 | 5.5 | 57 | 0.01 | 2.22 | 1 | 5.5 | 57 |
| *02.Sugar & sugar products including chocolate* | *401* | *78 %* | 0 | 0.1 | 0 | 0.4 | 0.4 | 0.1 | 0.18 | 0.1 | 0.4 | 0.4 |
| *03.Fats (vegetable and animal)* | *4* | *75 %* | 0 | 0.08 | 0 | 0.1 | 1.7 | 0.05 | 1.89 | 2.5 | 2.5 | 2.5 |
| 04B.Vegetables, nuts, pulses except vegetable soups | 47 | 77 % | 0 | 0 | 0 | 0.01 | 0.01 | 0.01 | 1.92 | 2.5 | 2.5 | 2.5 |
| *04.Vegetables, nuts, pulses including carrots, tomato and leafy vegetables* | *47* | *77 %* | 0 | 0 | 0 | 0.01 | 0.01 | 0.01 | 1.92 | 2.5 | 2.5 | 2.5 |
| 07B.Soft drinks with percentage of fruits lower than nectar, excl fruit juice | 13 | 85 % | 0 | 0 | 0 | 0 | 0.08 | 0.05 | 0.69 | 0.1 | 2.5 | 2.5 |
| *07.Fruit and vegetable juices, soft drinks and bottled water* | *13* | *85 %* | 0 | 0 | 0 | 0 | 0 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| *08.Coffee, tea, cocoa (expressed as liquid)* | *29* | *97 %* | 0 | 0.1 | 0 | 0.5 | 0.5 | 0.1 | 1.77 | 2.5 | 2.5 | 2.5 |
| 09B.Wine and substitutes | 4 | 100 % | 0 | 0 | 0 | 0 | 0 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| 09C.Other alcoholic beverages and substitutes | 2 | 0 % | 0 | 0 | 0 | 0 | 0 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| *09.Alcoholic beverages* | *6* | *67 %* | 0 | 0.03 | 0 | 0 | 1 | 0.05 | 2.36 | 2.5 | 2.5 | 2.5 |
| 10A.Meat and meat products and substitutes | 2 | 100 % | 0 | 2.01 | 0 | 0.4 | 259 | 0.01 | 2.94 | 0.22 | 2.5 | 259 |
| *10.Meat and meat products, offal* | *2* | *100 %* | 0 | 6 | 0 | 18 | 18 | 0.1 | 6.87 | 2.5 | 18 | 18 |
| *11.Fish and seafood* | *40* | *98 %* | 0 | 1.75 | 0 | 0.4 | 259 | 0.01 | 2.67 | 0.2 | 2.5 | 259 |
| 13A.Milk and dairy-based drinks | 277 | 85 % | 0 | 0.06 | 0 | 0.3 | 10 | 0.01 | 1.33 | 1 | 2.5 | 10 |
| 13B.Dairy-based products | 3 | 67 % | 0 | 2.55 | 0 | 10 | 50 | 0.05 | 3.84 | 2.5 | 10 | 50 |
| 13C.Cheese | 49 | 96 % | 0 | 0.84 | 0 | 1 | 50 | 0.01 | 2.11 | 2.38 | 2.5 | 50 |
| *13.Milk and dairy-based products* | *329* | *87 %* | 0 | 3.44 | 0 | 0 | 410 | 0.04 | 5.41 | 2.5 | 2.5 | 410 |
| 14A.Miscellaneous | 418 | 91 % | 0 | 0.03 | 0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| 14B.Food for special dietary uses | 190 | 71 % | 0 | 1.81 | 0 | 4.2 | 410 | 0.01 | 2.91 | 1 | 4.2 | 410 |
| *14.Miscellaneous / Food for special dietary uses* | *608* | *85 %* | 0 | 0.37 | 0 | 0.25 | 19.9 | 0.01 | 1.28 | 0.25 | 2.5 | 19.9 |
| *16.Feed* | *167* | *96 %* | 0 | 1.3 | 0 | 5.5 | 57 | 0.01 | 2.22 | 1 | 5.5 | 57 |
| *Not classified* | *3* | *67 %* | 0 | 0.1 | 0 | 0.4 | 0.4 | 0.1 | 0.18 | 0.1 | 0.4 | 0.4 |
| | *2239* | *80 %* | 0 | 0.08 | 0 | 0.1 | 1.7 | 0.05 | 1.89 | 2.5 | 2.5 | 2.5 |

[*)] The lower bound (LB) is obtained by assigning a value of zero (minimum possible value) to all the samples reported as <LOD or <LOQ. The upper bound (UB) is obtained by assigning the value of LOD to values reported as <LOD and LOQ to values reported as <LOQ (maximum possible value); n: number of samples; min: minimum value; P95: 95$^{th}$ percentile; max: maximum value.

### 4.4.2. Melamine occurrence data in food provided by industry

The CIAA, CEPE and Empac dataset included samples identified with generic or specific names and in most cases ad-hoc, not standardised, food categories. The data were coded according to the food groups of the Concise Food Consumption Database (EFSA, 2008b) and the occurrence statistics were calculated for the different food groups. When values were reported for concentrated or dried food items, dilution factors have been applied.

As explained previously, a large majority of the samples appear to be reported as Upper Bound values, therefore the few hundred samples reported as not detected were also attributed the value of their limit of detection. In some cases, when the limit of detection was not reported, it was arbitrarily given the

same value as contiguous products of similar type or origin. Table 5 presents the occurrence statistics for melamine in food according to the database from the industry.

**Table 5:** Statistics on occurrence of melamine in food (mg/kg) in categories of the Concise Food Consumption database from the CIAA, CEPE and Empac dataset.

| Concise Sub-category | Upper bound [1] (mg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | n | min | mean | median | P95 | max |
| 01A.Cereal-based mixed dishes | 5 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| 01B.Cereals & cereal products excl. cereal-based mixed dishes | 2016 | 0.01 | 0.07 | 0.05 | 0.25 | 0.6 |
| *01.Cereals & cereal products* | *2021* | *0.01* | *0.07* | *0.05* | *0.25* | *0.6* |
| *02.Sugar & sugar products including chocolate* | *1324* | *0* | *0.04* | *0.02* | *0.05* | *2.5* |
| *03.Fats (vegetable and animal)* | *134* | *0.05* | *0.06* | *0.05* | *0.1* | *0.5* |
| 04A.Vegetable soups | 46 | 0.01 | 0.03 | 0.01 | 0.1 | 0.1 |
| 04B.Vegetables, nuts, pulses except vegetable soups | 973 | 0.01 | 0.12 | 0.05 | 0.5 | 2.2 |
| *04.Vegetables, nuts, pulses including carrots, tomato and leafy vegetables* | *1019* | *0.01* | *0.12* | *0.05* | *0.5* | *2.2* |
| *05.Starchy roots or potatoes* | *60* | *0.01* | *0.06* | *0.05* | *0.06* | *0.5* |
| *06.Fruits* | *131* | *0.01* | *0.05* | *0.05* | *0.06* | *0.1* |
| 07A.Fruit and vegetable juices | 30 | 0.01 | 0.05 | 0.05 | 0.05 | 0.2 |
| 07B.Soft drinks with percentage of fruits lower than nectar, excl fruit juice | 1 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| *07.Fruit and vegetable juices, soft drinks and bottled water* | *31* | *0.01* | *0.05* | *0.05* | *0.05* | *0.2* |
| *08.Coffee, tea, cocoa (expressed as liquid)* | *207* | *0* | *0.01* | *0* | *0.05* | *0.05* |
| *09.Alcoholic beverages* | *3* | *0.05* | *0.05* | *0.05* | *0.05* | *0.05* |
| 10A.Meat and meat products and substitutes | 560 | 0.01 | 0.06 | 0.05 | 0.13 | 0.94 |
| 10B.Edible offal and offal products | 4 | 0.05 | 0.06 | 0.05 | 0.1 | 0.1 |
| *10.Meat and meat products, offal* | *564* | *0.01* | *0.06* | *0.05* | *0.13* | *0.94* |
| 11A.Seafood and seafood products | 47 | 0.01 | 0.03 | 0.02 | 0.05 | 0.05 |
| 11B.Fish and fish products | 136 | 0.01 | 0.06 | 0.05 | 0.1 | 0.6 |
| 11C.Fish-based preparations | 5 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| *11.Fish and seafood* | *188* | *0.01* | *0.05* | *0.05* | *0.1* | *0.6* |
| *12.Eggs* | *315* | *0.01* | *0.03* | *0.01* | *0.1* | *0.16* |
| 13A.Milk and dairy-based drinks | 8028 | 0 | 0.04 | 0.03 | 0.2 | 5.82 |
| 13B.Dairy-based products | 1184 | 0.01 | 0.06 | 0.01 | 0.2 | 0.64 |
| 13C.Cheese | 509 | 0.01 | 0.07 | 0.05 | 0.25 | 0.5 |
| *13.Milk and dairy-based products* | *9721* | *0* | *0.05* | *0.03* | *0.2* | *5.82* |
| 14A.Miscellaneous | 1704 | 0 | 0.06 | 0 | 0.30 | 9.48 |
| 14B.Food for special dietary uses | 993 | 0.01 | 0.03 | 0.01 | 0.1 | 0.34 |
| *14.Miscellaneous / Food for special dietary uses* | *2697* | *0* | *0.05* | *0.01* | *0.1* | *9.48* |
| *15.Tap water* | *20* | *0.01* | *0.05* | *0.05* | *0.15* | *0.2* |
| *Not classified* | *103* | *0.01* | *0.05* | *0.01* | *0.1* | *0.5* |
| | *18538* | *0* | *0.05* | *0.03* | *0.2* | *9.48* |

[1] A large majority of the samples appear to be reported as Upper Bound values (i.e. as their limit of detection); therefore the few hundred samples reported as not detected were also attributed the value of their limit of detection. n: number of samples; min: minimum value; P95: 95th percentile; max: maximum value.

Very few samples in the original database, before applying dilution factors, show high values, as reported in Table 6 which summarises the samples with melamine values above 10 mg/kg.

**Table 6:** Individual food samples in the CIAA, CEPE and Empac joint database with values of melamine exceeding 10 mg/kg.

| Ingredient/Product | Unit | Melamine level* |
|---|---|---|
| Ammonium bicarbonate | mg/kg | 948 |
| Ammonium bicarbonate | mg/kg | 542 |
| Milk powder | mg/kg | 58 |
| Milk powder | mg/kg | 23 |
| Milk powder | mg/kg | 23 |
| Ferric pyrophosphate | mg/kg | 20 |
| Ferric pyrophosphate | mg/kg | 19 |
| Ferric-pyrophosphate | mg/kg | 18 |
| Ferric pyrophosphate | mg/kg | 14 |
| Ferric pyrophosphate | mg/kg | 14 |

* In samples in dry form, before application of dilution factors

These values are few and appear to have an impact only on the maximum. The samples of ammonium bicarbonate (used in food processing) and milk powder appear to relate to specific incidents of adulteration and therefore are excluded from the calculations of background dietary exposure. The samples of ferric pyrophosphate are more likely to have been subject to cross-contamination and are therefore included in the exposure assessment (in the category 14A, miscellaneous).

### 4.4.3. Melamine occurrence data in feed provided by industry

The CIAA, CEPE and Empac dataset contained 510 occurrence data for melamine in feed. No detailed grouping for feed samples was provided, therefore only two groups were discriminated: cattle feed and other feed. The statistical descriptors for melamine occurrence according to these 2 groups are given in Table 7.

**Table 7:** Occurrence values for melamine (mg/kg) measured in feed categories.

| Feed Category | Upper bound (mg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | n | min | mean | median | P95 | max |
| Cattle feed | 145 | 0.05 | 18.18 | 5 | 5 | 860 |
| Other Feed | 357 | 0.05 | 0.09 | 0.05 | 0.5 | 0.86 |
| | 502 | 0.05 | 5.32 | 0.05 | 5 | 860 |

n: number of samples; min: minimum value; P95: 95$^{th}$ percentile; max: maximum value.

The number of data is limited, therefore the few samples with relatively high values, presented in Table 8, have an evident impact on the statistics.

**Table 8:** Individual feed samples with values of melamine exceeding 10 mg/kg in the CIAA, CEPE and Empac joint database.

| Feed Category | Unit | Melamine level |
|---|---|---|
| Animal Feed | mg/kg | 860 |
| Animal Feed | mg/kg | 516 |
| Animal Feed | mg/kg | 464 |
| Animal Feed | mg/kg | 180 |
| Animal Feed | mg/kg | 133 |
| Animal Feed | mg/kg | 13 |
| Animal Feed | mg/kg | 12 |

Based on the origin and date of the samples, it appears that these all related to a single incident. Therefore they are not representative of background levels and are excluded from the exposure assessment in animals. Table 9 shows the occurrence statistics after exclusion of these samples.

**Table 9:** Occurrence values for melamine (mg/kg) measured in feed categories, after exclusion of non-background values.

| Feed Category | Upper bound (mg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | n | min | mean | median | P95 | max |
| Cattle feed | 138 | 0.05 | 3.32 | 5 | 5 | 5 |
| Other Feed | 365 | 0.05 | 0.09 | 0.05 | 0.5 | 0.86 |
| | 503 | 0.05 | 0.98 | 0.05 | 5 | 5 |

n: number of samples; min: minimum value; P95: 95$^{th}$ percentile; max: maximum value.

### 4.4.4. Melamine levels in food and feed from food contact materials

4.4.4.1. Migration of melamine from food contact materials

The data from food surveys (sections 4.4.2 and 4.4.3) do not allow identification of the contribution from Food Contact Materials (FCMs). Furthermore, an important application of melamine–formaldehyde plastics is for domestic applications, which is not considered in retailed food surveys. Hence there is a need to consider separately the migration from food contact materials. The current section focuses on the migration of melamine.

There are three possible sources of exposure to melamine from food contact materials:

- tableware made of melaware;
- canned foods when the can coating contains melamine;
- adhesives and other miscellaneous food packaging applications.

4.4.4.1.1. Melaware

Tableware made of melaware are re-usable articles that may be used on a daily basis for many infants and children at household level and/or in kindergarten or school canteens. In the adult population such dishes may also be used on a daily basis at household level (e.g. plastic bowls or mugs) but would more typically be used on specific occasions such as camping or picnic.

Considering the use of melamine as a starting substance, one should pay attention in first instance to the potential migration into food of residues that may be present as a result of incomplete polymerisation. The chemical stability of the melamine-based materials must also be considered since degradation by e.g. hydrolysis may occur, especially in high temperature uses and possibly accelerated by acid.

Highest levels of migration of melamine might be expected to occur in the case of tableware in contact with acidic foods and beverages which are consumed hot. Possible cases would be tea with lemon, tomatoes soup, sweet sour Asiatic foods, apple purée, sauerkraut. Lower migration of melamine might be expected with acidic and non-acidic foods which are consumed cold.

**Food simulants used for testing plastics for migration**

Most migration testing of melaware has used 3% acetic acid as a food simulant (a simplified model food). Simulants intended to mimic the migration from plastics into foods were introduced in the early-1980's (Directive 82/711/EEC, as amended) along with the rules for using simulants such as the time and temperature test conditions to be applied (Directive 85/572/EEC, as amended). For melaware articles - as is the case for other plastic articles used at home - the conditions of use by the consumer are not known exactly and so rather severe test conditions are specified in the legislation. This

includes the requirement to test using three repeated exposures of the melaware to the simulant to try to indicate migration levels expected during the service-life of the article. Migration test results for such articles are frequently expressed on a contact area-related basis in units of mg/dm$^2$ where one dm$^2$ is 100 cm$^2$. When the ratio of surface contact area to food mass is known the results can be recalculated into units of mg/kg (mg of melamine per kg of food simulant). If the ratio is not know or if it is variable then a conventional surface area to mass ratio of 6 dm$^2$:kg is often used. The vast majority of melamine migration data come from compliance testing using acetic acid as a food simulant. Only recently have comparable data become available with migration levels for melamine into other (non-acidic) simulants and into foods themselves.

**Migration from melaware into acidic simulants**

Lund and Petersen (2002; 2006) tested 15 different items of kitchenware using 3% acetic acid. This was considered to be the most aggressive food simulant towards melaware. Most of the articles were tested for 2 hours at 70°C under repeated-use conditions. Generally, migration of both melamine and formaldehyde was measurable in the first or second test. In the subsequent tests, the migration most frequently dropped below the limit of quantification (ca. 1-2 mg/kg for each substance) but subsequently increased, so that measurable quantities were released in the tenth test. For all samples the migration was less than the legislative restrictions in force, which for melamine was the SML of 30 mg/kg food. The highest migration of melamine was about 5 mg/kg. There was no simple relationship between migration levels of formaldehyde (F) and melamine (M) and indeed the F:M; molar ratio changed from 12 down to 5. There was a high between-specimen variability (even for samples with the same batch code) which seems to be a general feature of migration from melaware articles. Lund and Petersen (2002; 2006) also tested 11 melaware cups that had been used already for several years in a day nursery. Migration of melamine into acetic acid simulant was not detectable for 8 of the samples (LOD ca. 1.5 mg/kg) and was ca. 2 to 3 mg/kg for the other 3 samples. The authors concluded that the results overall indicate that continuous migration of formaldehyde and melamine takes place during the lifetime of these articles. They concluded that the molar ratio of released formaldehyde to melamine indicates that, first, the migration of residual monomers is most important, but in the long-term, breakdown of the polymer dominates.

In a survey of 50 samples on the UK market (Bradley et al., 2005), migration of formaldehyde into acetic acid simulant was problematic but migration of melamine was always well below the SML then in force (30 mg/kg food). Each sample was tested three times using 3% acetic acid simulant for 2 hours at 70°C. Results from the first, second and third exposures were variable; for some samples the migration levels remained fairly constant but for other samples the migration increased or decreased. There was also very considerable variability between replicate specimens taken of the same type and brand name. Melamine migration was measurable for 43 of the 50 survey samples. The authors took the result for the third exposure as indicative of migration that may be expected during the service life of such repeat-use articles. Migration was in the range 0.3 to 5.4 mg/kg with a mean of 1.6 mg/kg. For some articles the melaware was observed to degrade during repeated exposure to simulant. The surface of the plastic became pitted and some cups even cracked and fell to pieces.

As part of checks made on imports in 2009, the Finnish Customs Laboratory tested 30 melaware plates and cups for formaldehyde migration into 3 % acetic acid for 2 hours at 70˚C (FFSA, 2010). The samples were all compliant for formaldehyde migration. The test solutions of 2 of the 30 samples were also measured for melamine using LC-MC/MS and melamine was not detected with an LOQ of 0.01 mg/kg.

In tests conducted in 2008 and 2009 in Cyprus, 23 samples of plates, cups and bowls were examined for migration of melamine using 3% acetic acid simulant and test conditions of 2 hours at 70ºC. All of the samples originated from China and they were tested by the State General Laboratory of Cyprus in Nicosia as part of their market control programme (SGL, 2010). The HPLC-UV method used had a LOQ of melamine of 0.025 mg/dm². Each specimen was tested three consecutive times. Twelve samples with duplicate specimens were tested in 2008 and the individual results for all three exposures

were reported. Figure 5 plots the 22 specimens for 11 samples in duplicate (the 12th sample is excluded from Figure 5, see later) to give an idea of the between-specimen variability and the trend from first to second to third exposure (diamonds, squares and triangles respectively). The highest result was 0.77 mg/dm$^2$ (equivalent to ca. 4.6 mg/kg) for the first exposure test of specimen A of sample 1. Ten of the twelve samples showed lower migration on the third exposure and the average of results for the third exposure was 0.29 mg/dm$^2$. This average was skewed by one high sample at 2.25 mg/dm² (not shown in Figure 5) and eliminating this sample lowered the average to 0.11 mg/dm² and the highest of the remaining samples was 0.38 mg/dm² a. For the samples tested in 2009 eleven samples with triplicate specimens were tested but only the result for the third exposure was reported. The average of the third exposure was 0.10 mg/dm² and the highest sample was 0.19 mg/dm².

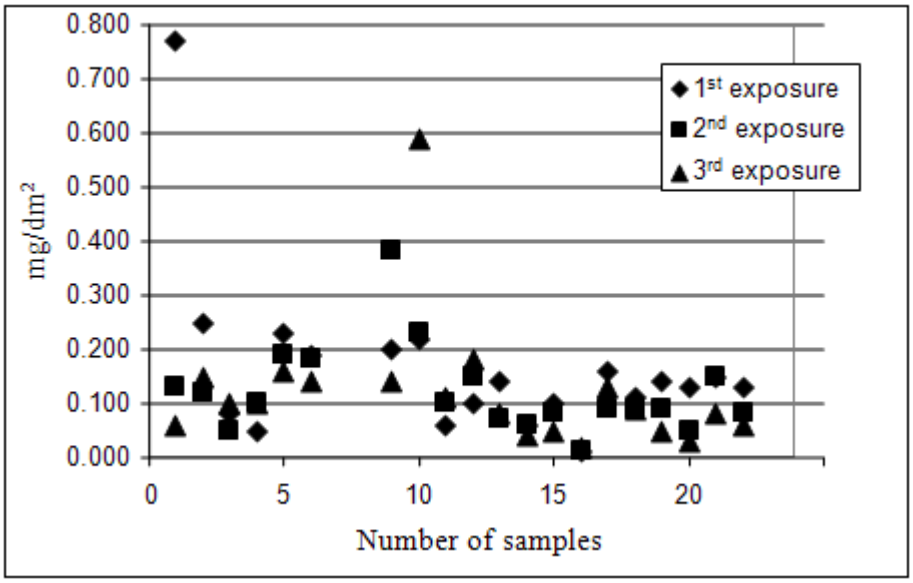



**Figure 5:** Migration of melamine in units of mg/dm² from 22 specimens (11 matched pairs) of melaware tested with 3 % acetic acid for 2 hours at 70ºC, showing between-specimen variability and the trend from first, second to third exposure to simulant (horizontal axis: specimen number).

**Migration from melaware into acidic and non-acidic simulants**

To investigate if 3 % acetic acid is an appropriate simulant for foods that are not especially acidic, twenty seven melaware articles were tested for migration of melamine into the food simulants 3 % acetic acid and distilled water using a variety of time and temperature test conditions (BfR, 2010a) (Table 10). The simulants were analysed using an LC-MS/MS method with an LOD of 5 µg/L. Duplicate specimens of 14 of the articles were tested - one with acetic acid and one with water using the same time and temperature conditions. Results in the table below are for the first migration exposure. A detailed comparison of the results point-by- point is not possible because no information is available on the between-specimen variability. However, a comparison of the ratio of the test result into water to the result for the acidic simulant shows that in 12 of the 14 pairs tested the water result is lower, at an average of 38 % of the acid result. Taken as a whole and allowing for possible between-specimen inhomogeneity, it seems that 3 % acetic acid generally elicits higher migration values than water, but the effect is not dramatic and seems to be on average a factor of two.

A number of other samples were tested using one of the simulants but not both. The results are presented at the foot of Table 10. From the whole data set it can be seen that the migration spanned a wide range, from the lowest at <0.005 up to the highest at 1.2 mg/kg of simulant.

**Table 10:** Migration of melamine from melaware articles into water in comparison with 3 % acetic acid (BfR, 2010a).

| Sample | Test conditions | Water mg/kg | Acetic acid mg/kg | Ratio Water/Acetic acid |
|---|---|---|---|---|
| Child's plate | 30 min 40°C | 0.020 | 0.160 | 0.13 |
| salad article | 30 min 40°C | 0.050 | 0.0013 | 3.85 |
| spatula | 10 min 70°C | 0.080 | 0.138 | 0.58 |
| kitchen aid | 10 min 70°C | 0.006 | 0.0010 | 0.60 |
| spoon | 10 min 70°C | 0.028 | 0.103 | 0.27 |
| whisk | 10min 70°C | 0.035 | 0.076 | 0.46 |
| bowl | 30 min 70°C | 0.183 | 0.926 | 0.20 |
| box | 30 min 70°C | 0.082 | 0.276 | 0.30 |
| plate | 30 min 70°C | 0.070 | 0.160 | 0.44 |
| plate | 30 min 70°C | 0.070 | 0.190 | 0.37 |
| plate | 120 min 70°C | 0.065 | 0.052 | 1.25 |
| spoon | 10 min 100°C | 0.090 | 0.740 | 0.12 |
| spoon | 10 min 100°C | 0.650 | 0.880 | 0.74 |
| plate, 1st test | 24h 40°C | 1.160 | not tested | - |
| plate, 3rd test | 24h 40°C | 0.230 | not tested | - |
| gravy ladle | 30 min 40°C | 0.010 | not tested | - |
| plate | 30 min 70°C | 0.093 | not tested | - |
| plate | 30min 70°C | 0.075 | not tested | - |
| plate | 30min 70°C | 0.128 | not tested | - |
| cup | 30 min 70°C | 0.150 | not tested | - |
| spoon | 120 min 70°C | 0.025 | not tested | - |
| soup ladle | 120 min 70°C | 0.270 | not tested | - |
| bowl | 30 min 70°C | not tested | 0.140 | - |
| beaker | 30 min 70°C | not tested | 0.050 | - |
| spatula | 10 min 100°C | not tested | 0.090 | - |

**Migration from melaware into acidic simulant and acidic beverages**

In a comparison exercise using the acid food simulant 3% acetic acid and 5 types of beverages, 6 large cups (500 mL, inner surface area: 280 $cm^2$) and 6 small cups (200 mL, inner surface area: 160 $cm^2$) were tested each with a first second and third exposure to one of the 6 liquids (BfR, 2010b). Before testing the cups were washed as in domestic use. After the migration period, melamine extraction and sample clean-up was by solid phase extraction using a cation-exchange resin followed by isotope-dilution ($^{13}C_3$-melamine) LC-MS/MS analysis. The LOQ was about 3.5 µg/L of beverage or food simulant and the results are summarised in Table 11.

**Table 11:** Migration of melamine (µg/L) into acidic beverages and the ratio (%) of migration compared to 3 % acetic acid (BfR, 2010b).

| | 3 % acetic acid | apple juice | tomato juice | tea made of red fruits | coffee | cola drink |
|---|---|---|---|---|---|---|
| **test conditions** | **2 h/70°C** | **2 h/70°C** | **2 h/70°C** | **2 h/70°C** | **2 h/70°C** | **2 h/RT[1]** |
| pH | 2.56 | 3.43 | 4.22 | 3.58 | 5.03 | 2.61 |
| Large cups | | | | | | |
| 1st (µg/L, ratio) | 35 | 12 , 35 % | 60 , 174 % | 17.6 , 51 % | 44 , 127 % | 2.4 , 7 % |
| 2nd (µg/L, ratio) | 92 | 24 , 26 % | 168 , 183 % | 24 , 26 % | 23 , 25 % | 0.23 , <1 % |
| 3rd (µg/L, ratio) | 112 | 20 , 17 % | 195 , 174 % | 18 , 160 % | 21 , 18 % | 0.8 , <1 % |
| Small cups | | | | | | |
| 1st (µg/L, ratio) | 631 | 467 , 74 % | 439 , 70 % | 121 , 19 % | 707 , 112 % | 2.2 , <1 % |
| 2nd (µg/L, ratio) | 836 | 1132 , 135 % | 843 , 101 % | 172 , 21 % | 658 , 79 % | 1.3 , <1 % |
| 3rd (µg/L, ratio) | 559 | 1192 , 213 % | 865 , 155 % | 155 , 28 % | 458 , 82 % | 0.7 , <1 % |

[1] RT = room temperature

The results should not be over-interpreted because no information is available on the between-specimen variability - these are results for 3 repeat tests on single specimens. Nevertheless, it is informative to calculate the migration into the 5 different foodstuffs as a ratio to the migration into the acid simulant (Table 11). The results for the apple juice, tomato juice, red-fruit tea and coffee seem to be scattered around the results for the acetic acid simulant - some higher and some lower - giving some evidence that the simulant adequately represents low pH beverages when the same time and temperature test conditions are used. On the other hand, despite the low pH of the cola, being the closest in pH to the acetic acid simulant, the much lower migration into cola suggests a very strong effect of temperature since the cola alone was applied at room temperature, whereas the other tests were using the test liquid preheated and held in the cups at 70°C for 2 hours.

**Migration of melamine into beverages and into a variety of food simulants**

In a report submitted to EFSA by The Netherlands Organisation for Applied Scientific Research - TNO (TNO, 2010) three types of melaware cups were purchased in the Netherlands in March 2010 and tested for the migration of melamine into a variety of simulants and foods. Cup brand 1 was white with a capacity of 200 ml. Cup brand 2 was coloured with a capacity of 250 ml. Cup brand 3 was white with a printed design and had a capacity of 200 ml.

The food or simulant was pre-warmed and then used to fill the cups. The cups were labelled with the advice not to use in microwave ovens. The instruction was embossed permanently in the plastic. To see what would be the migration if the user ignored such instructions, microwave heating was used where the cup was filled with cold food/simulant, brought to boiling using a microwave oven, and then placed in a 40°C oven for 24 hours. The heating times and the wattage of the oven were not given. Some of the cups could not withstand microwaving three times and they cracked. These are indicated in the tables below.

For each migration test listed in Table 12 new cups were used and duplicate cups were each exposed three times to food/simulant. After the migration period the food or simulant was analysed for melamine by LC-MS. The results for the 1st and 3rd exposures are given in Table 12 along with the duplicate results (A and B) to indicate the between-specimen homogeneity.

The results confirmed the strong influence of temperature on migration. Migration into 3 % acetic acid, water or 50 % ethanol was much higher at 70°C than at 40°C. Migration after microwaving was

comparable to the testing with 3 % acetic acid for 2 hours at 70°C. However, there was no detectable migration into the fatty food simulant olive oil even under test conditions of 2h at 70°C. In one set of tests the inner surface of the cups was scratched deliberately before testing with 3 % acetic acid and microwave heating. Although the melamine migration was higher for the scratched samples, they did survive the three repeated exposures whereas both of the unscratched samples cracked and could not be tested a third time, so a firm conclusion on the effect of surface damage could not be drawn.

**Table 12:** Migration of melamine (mg/kg) from melaware cups into beverages and into a variety of food simulants (TNO, 2010).

| Food/simulant | Test condition | 1st migration mg/kg | | | 3rd migration mg/kg | | |
|---|---|---|---|---|---|---|---|
| | | A | B | Avg | A | B | Avg |
| **BRAND 1** | | | | | | | |
| 3 % acetic acid | 2h at 70°C | 1.815 | 1.556 | 1.686 | 1.703 | 1.494 | 1.599 |
| 50 % ethanol | 2h at 70°C | 0.303 | 0.440 | 0.371 | 0.093 | 0.103 | 0.989 |
| olive oil | 2h at 70°C | < 0.026 | | | | | |
| 3 % acetic acid | 24 h at 40°C | <0.051 | 0.256 | 0.301 | 0.279 | | |
| 50 % ethanol | 24 h at 40°C | 0.040 | 0.040 | 0.040 | 0.033 | 0.031 | 0.032 |
| olive oil | 24 h at 40°C | < 0.026 | | | | | |
| water | 24 h at 40°C | 0.027 | < 0.026 | 0.027 | 0.036 | < 0.026 | 0.031 |
| milk | 24 h at 40°C | < 0.026 | 0.034 | 0.030 | 0.045 | 0.061 | 0.053 |
| cola | 24 h at 40°C | 0.037 | 0.075 | 0.056 | 0.492 | 0.225 | 0.359 |
| orange juice | 24 h at 40°C | < 26 | 0.029 | < 0.026 | 0.028 | | |
| 3 % acetic acid | Microwave +24h/40C | 0.607 | 3.326 | 1.966 | 2.505 | 5.882 | 4.194 |
| orange juice | Microwave +24h/40C | 1.457 | 0.666 | 1.062 | 1.711 | 1.187 | 1.449 |
| **BRAND 2** | | | | | | | |
| 3 % acetic acid | 2h at 70°C | 0.342 | 0.427 | 0.385 | 0.334 | 0.328 | 0.331 |
| 50 % ethanol | 2h at 70°C | 0.085 | 0.173 | 0.129 | 0.056 | 0.055 | 0.056 |
| olive oil | 2h at 70°C | | | <0.026 | | | |
| 3 % acetic acid | 24 h at 40°C | <0.051 | 0.201 | <0.051 | 0.201 | <0.051 | 0.201 |
| 50 % ethanol | 24 h at 40°C | | | <0.026 | | | |
| olive oil | 24 h at 40°C | | | <0.026 | | | |
| water | 24 h at 40°C | 0.076 | 0.079 | 0.078 | 0.087 | 0.092 | 0.089 |
| milk | 24 h at 40°C | | <0.026 | | 0.0416 | <0.026 | 0.034 |
| cola | 24 h at 40°C | 0.046 | 0.064 | 0.055 | 0.121 | 0.167 | 0.144 |
| orange juice | 24 h at 40°C | | | <0.026 | | | |
| 3 % acetic acid | Microwave +24h/40C | 0.781 | 0.546 | 0.664 | cracked | cracked | - |
| orange juice | Microwave +24h/40C | 0.377 | 0.136 | 0.257 | 0.612 | 0.481 | 0.547 |
| milk | Microwave +24h/40C | 0.560 | 0.251 | 0.406 | 1.449 | 0.174 | 0.812 |
| 3 % acetic acid | Microwave +24h/40C (scratched) | 1.347 | 701 | 1.024 | 1.602 | 1.014 | 1.308 |
| **BRAND 3** | | | | | | | |
| 3 % acetic acid | 2h at 70°C | 1.089 | 1.101 | 1.095 | 0.585 | 0.690 | 0.638 |
| 50 % ethanol | 2h at 70°C | 0.190 | 0.141 | 0.166 | 0.070 | 0.052 | 0.061 |
| olive oil | 2h at 70°C | | | <0.026 | | | |
| 3 % acetic acid | 24 h at 40°C | 0.078 | 0.087 | 0.082 | 0.334 | 0.293 | 0.314 |
| 50 % ethanol | 24 h at 40°C | 0.055 | 0.042 | 0.048 | 0.045 | 0.028 | 0.037 |
| olive oil | 24 h at 40°C | | | <0.026 | | | |
| water | 24 h at 40°C | 0.049 | 0.038 | 0.043 | 0.027 | 0.029 | 0.028 |
| milk | 24 h at 40°C | 0.177 | 0.054 | 0.116 | 0.119 | 0.029 | 0.074 |
| cola | 24 h at 40°C | 0.130 | 0.110 | 0.120 | 0.257 | 0.394 | 0.326 |
| orange juice | 24 h at 40°C | 0.042 | 0.053 | 0.048 | 0.040 | 0.026 | 0.033 |
| 3 % acetic acid | Microwave +24h/40C | 1.275 | 1.675 | 1.475 | cracked | cracked | - |
| orange juice | Microwave +24h/40C | 1.244 | 0.795 | 1.020 | cracked | cracked | - |

**Migration of melamine and analogues into foods and food simulants**

In complementary experiments, individual melaware articles were exposed successively to simulant, food, simulant (or vice-versa) to eliminate the problem of between-article variability in the interpretation of results. The articles (5 replicate specimens of each with the same batch code) were purchased and tested in 2010 (FERA, 2010). They were a bowl and a cup and were highly decorated as for child's use. They were washed in warm soapy water and dried before use, as per the label instructions. There were instructions on a self-adhesive label, advising against microwave use. To investigate what the migration would be if the user lost or ignored such instructions, some microwave heating experiments were conducted too. For filling with liquids (simulant and juice) the bowls required 220 mL and cups required 250 mL, and the contact areas were 1.52 $dm^2$ and 1.98 $dm^2$ respectively. For filling with food, 163 g was placed in the bowls and the surface area covered was 1.22 $dm^2$.

The food simulant was 3% acetic acid applied for 2 hours at 70°C. In the hot fill experiments, the cup or bowl was filled with pre-heated food and then left to stand at room temperature for 2 hours. Two different foodstuffs were used and they were pre-heated to boiling using a glass bowl in a microwave oven and then poured into the melaware. In the microwave heating experiments, the food was heated in the melaware article (2 min at 600W nominal output for spaghetti hoops and sausages, 3.3 min at 600W until boiling for juice) and then left to stand at room temperature for 2 hours. In this way, the hot-fill tests mimicked using the articles to serve and feed a child whereas the microwaving experiments mimicked using the melaware as cookware (for heating) and then as service ware for feeding.

The exposed foods and food simulant samples were analysed along with blanks, for melamine, cyanuric acid, ammeline and melamine using an LC-MS/MS method. $^{13}C_3$-melamine was used as the internal standard and recovery checks were conducted using blank samples spiked at 0.02 and 2 mg/kg of each of the 4 analytes.

The results for melamine migration are shown in Table 13. Melamine migrated at measurable concentrations into the hot 3% acetic acid simulant from both types of article in all exposures. Migration was constant (Bowl 1) or declined (Cup 1) for the 3 successive exposures to hot simulant. Melamine migrated into spaghetti hoops and sausages when the food was microwave-heated in the bowl and the migration level was broadly similar (and perhaps a little lower) to the simulant results (Bowls 2 and 3). It seems likely that, although they will have cooled quickly on standing, the peak temperature achieved on microwaving will have exceeded 70°C and this higher temperature counterbalances the shorter time held hot compared to the 2 hours at 70°C simulant tests. In contrast, migration was not measurable when the bowl was filled with hot food (Bowls 4 and 5) and assuming migration was at the LOD, the results were thus at least 8 to 14-times lower than the simulant results. Similarly, melamine migrated into apple juice when microwave-heated to boiling in the cup (Cups 2 and 3) and again at levels comparable (and perhaps a little lower) to the simulant results. When the cup was hot-filled with boiling apple juice then migration of melamine was only detectable from the first exposure (Cup 5) and otherwise using the LOD, the migration was about 6-times lower than into simulant.

There was no measurable migration of cyanuric acid, ammeline or ammelide from any of the articles under any test conditions. The detection limits for these compounds varied by substance and matrix and were in the range of 0.003 to 0.065 mg/kg.

**Table 13:** Melamine migration into foods and simulants from melaware articles exposed in sequence (FERA, 2010).

| Article | Exposure | Food or simulant and conditions used | mg/kg |
|---|---|---|---|
| Bowl 1 | 1st | 3 % acetic acid, 2 hrs/70°C | 1.86 |
| | 2nd | 3 % acetic acid, 2 hrs/70°C | 1.43 |
| | 3rd | 3 % acetic acid, 2 hrs/70°C | 1.75 |
| Bowl 2 | 1st | 3 % acetic acid, 2 hrs/70°C | 1.72 |
| | 2nd | Spaghetti & sausages in tomato sauce, microwaved | 1.21 |
| | 3rd | 3 % acetic acid, 2 hrs/70°C | 1.82 |
| Bowl 3 | 1st | Spaghetti & sausages in tomato sauce, microwaved | 1.93 |
| | 2nd | 3 % acetic acid, 2 hrs/70°C | 2.71 |
| | 3rd | Spaghetti & sausages in tomato sauce, microwaved | 0.51 |
| Bowl 4 | 1st | 3 % acetic acid, 2 hrs/70°C | 2.02 |
| | 2nd | Spaghetti & sausages in tomato sauce, hot filled | <0.14 |
| | 3rd | 3 % acetic acid, 2 hrs/70°C | 1.90 |
| Bowl 5 | 1st | Spaghetti & sausages in tomato sauce, hot filled | <0.14 |
| | 2nd | 3 % acetic acid, 2 hrs/70°C | 1.10 |
| | 3rd | Spaghetti & sausages in tomato sauce, hot filled | <0.14 |
| Cup 1 | 1st | 3 % acetic acid, 2 hrs/70°C | 4.60 |
| | 2nd | 3 % acetic acid, 2 hrs/70°C | 2.63 |
| | 3rd | 3 % acetic acid, 2 hrs/70°C | 2.56 |
| Cup 2 | 1st | 3 % acetic acid, 2 hrs/70°C | 2.39 |
| | 2nd | Apple juice, microwaved | 1.27 |
| | 3rd | 3 % acetic acid, 2 hrs/70°C | 1.91 |
| Cup 3 | 1st | Apple juice, microwaved | 2.00 |
| | 2nd | 3 % acetic acid, 2 hrs/70°C | 1.88 |
| | 3rd | Apple juice, microwaved | 1.07 |
| Cup 4 | 1st | 3 % acetic acid, 2 hrs/70°C | 2.42 |
| | 2nd | Apple juice, hot filled | <0.34 |
| | 3rd | 3 % acetic acid, 2 hrs/70°C | 1.66 |
| Cup 5 | 1st | Apple juice, hot filled | 2.77 |
| | 2nd | 3 % acetic acid, 2 hrs/70°C | 1.58 |
| | 3rd | Apple juice, hot filled | <0.34 |

**Migration from melaware into foods**

In studies performed in Japan and the Philippines, both new (Sugita et al., 1990; Martin et al., 1992) and used melaware samples collected from canteens after several years of use (Ishiwata et al., 1986) were examined for migration into simulants and foods. The migration of melamine was in the range 0.5 to 2.2 mg/kg food in certain beverages kept hot in cups. The authors noted that migration levels tended to be higher into acid foods or beverages and also was higher at high contact temperatures. The behaviour on repeated testing of the same specimens gave mixed results. In some cases migration levels declined, whereas in other cases the migration levels increased. It was noted that for some samples the surface of the plastic seemed to be attacked and suffer deterioration such as roughening and tarnishing, especially when a solution of 3 % acetic acid was used as a food simulant. This surface roughening is also reported for melaware that has been used for several years (Ishiwata et al., 1986; Lund and Petersen, 2002, 2006).

In data provided to EFSA (ISAN, 2010) three brands of melaware dishes and three brands of cups, all intended for infants, were purchased in Piacenza (Italy) and tested for migration. The foods used were UHT whole milk (pH 6.58), vegetable soup (20 g of dehydrated solids dissolved in 1 L of distilled water, pH 6.03) and tannin-free tea (72 g of dehydrated solids dissolved in 1 L of distilled water, pH 6.44). The articles were filled with the food to within 5 mm of the rim at 60°C, 60°C and 80°C respectively, and then allowed to stand at room temperature for 2 hours after which time all samples

had cooled to a final temperature of about 21°C. The different temperature of milk, vegetable soup and tea were said to be chosen to simulate the real use of these foods. The capacity of the cups was 300, 350 and 250 mL for brands A-C respectively and the dishes had a capacity of 200, 130 and 200 mL respectively. Tests were conducted in triplicate. The exposed foods were tested for melamine content according to the extraction and LC-MS/MS procedure of Shia et al. (2008) with some modifications. As showed in the Table 14, migration into tannin-free tea was higher (63 to 105 µg/L) compared to the other two food types (2 to 19 µg/L) and this could be due to the higher temperature of tea at the beginning of the contact period.

**Table 14:** Migration of melamine (µg/L, mean ± SE) from baby food dishware into milk hot-filled at 60°C, vegetable soup filled at 60°C and tannin-free tea filled at 80°C, and allowed to cool over 2 hours. (n = 3) (ISAN, 2010).

| | Article | Time of contact | Brand A | Brand B | Brand C |
|---|---|---|---|---|---|
| Vegetable soup 60°C cooling to ambient | Dish | 120 min | 19 ± 13 | 2 ± 0.3 | 10 ± 3 |
| Whole milk 60°C cooling to ambient | Cup | 120 min | 12 ± 2 | 10 ± 2 | 8 ± 1 |
| Tannin free tea 80°C cooling to ambient | Cup | 120 min | 105 ± 61 | 63 ± 19 | 89 ± 39 |

**Conclusions on migration from melaware**

Visual inspection of melaware articles reveals that some have a glossy and seemingly hard surface whilst others have a matt appearance that seems to be softer. Some brands are labelled with instructions advising against microwave oven use and washing in automatic dishwashers whereas other brands can be found in the shops that lack one or both of these instructions, or the instructions are not permanently embossed in the article but are on the packaging or on a label that can be lost and the instructions forgotten by the user.

According to Bradley et al. (2005) melaware articles are made by compression moulding of melamine-formaldehyde resin from powder or granular form. The heat and the pressure of the moulding process cures the resin to provide a thermoset plastic. Most moulding powders contain additives such as cellulosic fibres, pigments and fillers. It can be anticipated that poor formulations (inadequate formaldehyde to melamine ratio, presence of fillers) and/or poor process conditions (inadequate time and pressure of moulding) may yield articles showing very high migration values. However, migration levels are moderate to low for melaware articles that have a good quality surface finish.

Nevertheless, migration of melamine from melaware is characterised by a high between-specimen variability for some samples and by inconsistent behaviour on repeated contact with food or simulants with migration levels falling for the majority of samples but rising for others. A further complication is that the articles can have a long service life during which time the surface may become scratched. Migration clearly depends on the time and temperature conditions of use along with the characteristics of the food - acidic, aqueous, fatty or dry. In deriving migration values to be used in estimating exposure, a weight of evidence approach has been taken to arrive at estimates of *Typical migration values* and *High migration values* for the different food classes.

*Typical migration value* - a conservative estimate of the migration level for a melaware article that has a high migration potential and is placed in contact with hot foodstuffs using typical contact conditions of time and temperature.

*High migration value* - a conservative estimate of the migration level for a melaware article that has a high migration potential and is tested using food simulants that are applied according to regulations

using the worst foreseeable combination of time and temperature test conditions. These severe test conditions are considered to be equivalent to microwave oven heating and they are also considered to cover the possible scratching of articles during their service life.

Most of the migration data available for melaware is for the food simulant 3% acetic acid and only in recent months has any substantial data become available for other food simulants and for food and beverages themselves. For the simulant 3% acetic acid using high temperature test conditions with both new and old (used) melaware (Ishiwata et al. 1986; Lund and Petersen 2002, 2006; Bradley et al., 2005; SGL, 2010) and for acidic foods that are heated in melaware by microwave (FERA, 2010; TNO, 2010) migration up to about 5 mg/kg is observed. For hot-fill applications of cups and bowls with acidic foods migration was about 6-time lower than into acetic acid (FERA, 2010) so a typical migration value of 1 mg/kg is taken. For high temperature testing using water as a food simulant, migration was about half the levels seen into acetic acid (BfR, 2010a) and so a high migration value of 3 mg/kg is assumed. A corresponding value for hot-fill with non-acidic foods is taken to be one-fifth of this at 0.6 mg/kg. Based on polarity and solubility considerations, melamine is not expected to migrate at high levels into fatty foods and the very limited data available (TNO, 2010) confirm this with no detectable migration (<0.026 mg/kg) into olive oil simulant even using high temperature conditions. However, since fatty foods can contain both fat and water phases a high migration value of 1 mg/kg and a typical migration value of 0.2 mg/kg is assumed. For dry foods, migration of melamine is expected to be insignificant and a figure of 0.05 mg/kg is assumed for both typical and high values, using the miscellaneous background data (Table 15) (CIAA, CEPE and EMPAC, 2009).

**Table 15:** Estimated melamine migration values for each food class.

| Melamine migration | Melamine migration values (mg/kg) | |
|---|---|---|
| **Food class** | **Typical** | **High** |
| Acidic foods | 1.0 | 5.0 |
| Aqueous foods | 0.6 | 3.0 |
| Fatty foods | 0.2 | 1.0 |
| Dry foods | 0.05 | 0.05 |

Regarding the analogues cyanuric acid, ammeline and ammelide, only FERA (2010) tested for these three substances from melaware and only two brands of melaware were tested. No migration was detected, with detection limits in the range of 0.003 to 0.065 mg/kg. Since they are not authorised for use in plastics, it is assumed that melaware is not a source of food contamination by cyanuric acid, ammeline and ammelide.

4.4.4.4.2. Coatings on metal for cans and closures

**Migration of melamine and its analogues from coated light metal packaging into food simulants**

Migration data were provided to EFSA in February 2010 by a joint industry group (JIG) TSC31 comprising member companies of CIAA, EMPAC, CEPE and the principal manufacturers of melamine-based coating resins. According to this JIG, they set up a sampling and test protocol to gather melamine migration data from a representative cross-section of real, commercially-coated metal packaging. Many cans typically consist of a number of different coatings on different components, or parts of the can, for the can ends, bodies and side stripes. Coatings are also used on the metal closures for jars and bottles. The coatings tested were sourced from within the supply chain and based on epoxyanhydride or epoxyphenolic resins mixed with melamine resins, that were said to be representative of about 80 % of the current market for 2-piece and 3- piece food cans and closures for jars. They stated that very few, if any, beverage cans or ends use internal coatings containing melamine-based resins. Furthermore, food cans are processed at a higher temperature than beverage cans which would be important if any melamine may be generated by hydrolysis of the coating at high

food processing temperatures. The samples tested were commercial, coated metal packaging components and included coated cans, ends and closures for glass jars and each sample tested comprised one or more food-contact coatings.

The coatings were extracted using simulants under realistic industrial heat-processing conditions for canned and jarred foods. The food simulants and the retort conditions used were 10 % ethanol for 1hr at 130ºC (denoted E) and 3 % acetic acid for 1hr at 100ºC (denoted A). According to the JIG, to comply with EU anti-cartel rules governing such joint-industry projects, the exposed simulants were coded anonymously by an independent party (D. Smith, Technical manager, MPMA) and submitted blind to 6 participating laboratories for analysis who then reported results back to the coordinator. The identity of the 6 laboratories was provided to EFSA but they are simply coded 1 to 6 here.

The analytical results for melamine are shown in Table 16. The first 16 entries are grouped into 8 pairs to indicate that for each pair the same coating system was extracted separately with 3 % acetic acid (A) and with 10 % ethanol (E). Note that, because the samples were coded anonymously, each of the 8 pairs has 2 sample codes. The remaining 5 coating systems were extracted with 10 % ethanol only.

**Table 16:** Migration of melamine (mg/kg) from commercial coatings on light metal packaging (cans, closures) - TSC31 Analytical Results.

| LOD (mg/kg) | Lab 1 | Lab 2 | Lab 3 | Lab 4 | Lab 5 | Lab 6 |
|---|---|---|---|---|---|---|
| | 1 | 1.5 | 1 | 10 | 50 | 30 |
| **Can coatings** | | | | | | |
| 1A | 0.002 | 0.0035 | 0.002 | 0.016 | ND | ND |
| 16E | 0.001 | ND | ND | ND | ND | ND |
| 2A | 0.002 | 0.0017 | ND | ND | ND | ND |
| 3E | 0.001 | 0.0015 | ND | ND | ND | ND |
| 10A | 0.002 | ND | ND | ND | ND | ND |
| 5E | 0.010 | 0.011 | 0.009 | 0.032 | ND | ND |
| 6A | 0.008 | 0.0068 | 0.006 | 0.015 | ND | ND |
| 18E | 0.130 | 0.151 | 0.167 | 0.139 | 0.160 | 0.132 |
| 20A | 0.001 | ND | ND | ND | ND | ND |
| 7E | 0.003 | 0.003 | ND | ND | ND | ND |
| 9A | 0.002 | ND | ND | ND | ND | ND |
| 21E | 0.011 | 0.012 | 0.011 | 0.014 | < 0.070 | ND |
| 15A | 0.088 | 0.102 | 0.146 | 0.108 | 0.090 | 0.090 |
| 11E | 0.289 | 0.332 | 0.378 | 0.307 | 0.330 | 0.292 |
| 13A | 0.016 | 0.014 | 0.020 | 0.020 | ND | ND |
| 17E | 0.133 | 0.146 | 0.164 | 0.130 | 0.140 | 0.131 |
| **Closures e.g. jar lids** | | | | | | |
| 4E | 0.294 | - | 0.343 | 0.329 | 0.340 | 0.327 |
| 8E | 0.046 | 0.050 | 0.056 | 0.051 | 0.050 | 0.042 |
| 12E | 0.087 | 0.095 | 0.135 | 0.093 | 0.090 | 0.086 |
| 14E | 0.189 | 0.210 | 0.281 | 0.190 | 0.260 | 0.185 |
| 19E | 0.003 | 0.0025 | ND | ND | ND | ND |

E = 10 % ethanol 1hr/130ºC). A = (3 % acetic acid 1hr/100ºC. Sample codes 1A, 2A, 3E and 16E are metal closures such as those used to close glass jars for baby foods: The other samples were filled metal cans. LOD: limit of detection.

The results obtained from the participating laboratories show in general a good correlation bearing in mind that, according to the JIG, a range of analytical methods were used. Method details were not provided. Since the exercise was conducted anonymously the results cannot be interpreted in detail according to the different coating compositions, what melamine-formaldehyde resins they may contain, the stoving (curing) process used etc. All the migration results for melamine were below 0.5 mg/kg. From the results it can be concluded that for the coatings that do have melamine migration,

levels are significantly higher into 10 % ethanol (1hr/130ºC) compared to 3 % acetic acid (1hr/100ºC) suggesting a more important effect of temperature rather than acidity in causing migration.

*Cyanuric acid.* Two laboratories tested the simulant samples for cyanuric acid. All samples showed non-detectable levels at LODs of 0.001 mg/kg (Lab 1) or 0.020 mg/kg (Lab 2).

*Ammeline and ammelide.* Lab 2 also tested the samples for ammeline and ammelide with an LOD of 0.005 mg/kg for each substance. All samples were less than the LOD except for sample 11E which contained ammeline at 0.0077 mg/kg.

According to the JIG, the data indicates that migration levels of melamine can exceed the residual content in the can coating, as determined by solvent extraction. Thus, some decomposition of the coating can occur, probably by a hydrolysis mechanism, to form additional melamine. Although the data on can coatings and closures are limited, there seems to be a strong influence of the time and especially the temperature of heat treatment applied in the experiments with foods or simulants. Canned foods are pasteurised or sterilised using different combinations of time and temperature in the retorting process. Additionally, a relatively few types of coatings have been tested and their exact composition is unknown. Therefore, since all migration values into food and simulants are below 0.5 mg/kg, this concentration is used in the exposure estimates as a conservative assumption for the migration of melamine from coatings on cans and closures.

4.4.4.4.3. Adhesives and miscellaneous uses of melamine in food contact materials

**Adhesives**

The Joint Research Centre (JRC) (Ispra) provided EFSA with migration data from tests on 5 plastic laminates. The samples had been provided to the JRC by industry as candidate reference materials to be used in collaborative trials of melamine test methodology. No compositional information was provided - the laminates were described by industry as "may contain melamine". The food simulant used for the migration tests was 3 % acetic acid and the test conditions used were 2 hours at 70°C and at 100°C. At 70°C, 4 of the 5 samples gave no detectable migration of melamine (<0.06 mg/kg) whereas the fifth sample gave 0.19 mg/kg. At 100°C, 4 samples were in the range 0.14 to 0.36 mg/kg and the fifth sample was again by far the highest with melamine migration at 1.5 mg/kg. Since it is not known what were the intended use(s) of these laminates in contact with foods, it is not known if these high temperature test conditions and hence the test results, are appropriate or not.

**Additives for paper**

In a survey of retail paper and board food contact samples for residual amines including melamine, none of the 260 samples tested contained any extractable melamine. The detection limit of the extraction and analysis method used was 0.1 mg melamine per kg paper. For a typical paper weight of 2 g/dm$^2$ this equates to a worst-case migration potential of <0.001 mg/kg food (MAFF, 1996). More recently, in 2006, 12 retail samples of paper food contact materials were tested. None contained any extractable melamine and the migration potential was less than 0.01 mg/kg food. These results suggest that wet-strength agents used in paper making contain very little if any residual free melamine as used and/or that any residues that there are, are not fibre-retentive but are washed out during the paper-making process which employs large quantities of water to suspend the paper fibres (FSA, 2006).

**Melamine gas barrier coatings**

Four plastic films with a very thin layer of melamine sandwiched within different plastics/ink/adhesive structures (4 or 5 layers in total) were tested for the migration of melamine. The plastic films used on the two outside surfaces of each laminate were all about 15 µm thick. The food simulants used were water, 3 % acetic acid, 10 % ethanol and 50 % ethanol. The test conditions used were total immersion in the simulant at 40°C for 10 days. The surface to volume ratio was approximately 10 dm$^2$/kg. Two of the films gave no detectable migration into any simulant with a detection limit of 0.1 mg/kg. Two

films gave detectable migration into the acidic simulant, at 0.12 mg/kg and 0.24 mg/kg, and no detectable migration into the other 3 simulants used (DSM, 2009).

**Kitchen worksurfaces**

One application of melamine-formaldehyde plastics is the thick and durable coatings on wood or wood composites used as kitchen worksurfaces for food preparation. Such a coating was tested by total immersion in 3 % acetic acid for 2 hours at 40°C and there was no detectable migration of melamine (< 0.15 mg/dm$^2$) on each of three successive tests (NRL-FCM, 2010). Similarly, but using a different food simulant and test conditions, a worksurface coating was tested once using 95 % ethanol for 24 hours at 40°C and no migration of melamine was detected (< 0.07 mg/dm$^2$) (FhILV, 2010).

**Miscellaneous uses**

Industry (CIAA, CEPE and EMPAC, 2009) provided data on 430 miscellaneous packaging samples tested for melamine. The packaging materials were not described in detail and it is assumed that they were a mixture of plastics, paper/board etc. in the finished state and therefore made using adhesives etc. as required. The results from the packaging analysis were transformed into calculated maximum migration levels assuming total mass transfer and a packaging to food ratio of 6 dm$^2$ per kg. By these calculations, 340 samples were less than 6 µg/kg, 63 samples in the range 6-60 µg/kg, and 31 samples in the range 60-600 µg/kg. Consequently, the P90 value of migration potential was about 50 µg/kg.

### 4.4.5. Background level from cyromazine use

There are over 1,000 active substances (plus degradation products and metabolites) that could be applied to crops throughout the world. Only those considered of most importance are monitored. Cyromazine has not been included in the list of target pesticides in the EU harmonised monitoring programme. However, in light of the contamination of milk from China, some countries have included cyromazine in their national monitoring programmes. For example, cyromazine was added to the UK surveillance suite of pesticides for 2009. However, melamine was not included as it is not a pesticide and is not part of the MRL definition of cyromazine.

Cyromazine is also used in the treatment of poultry feed to control diptera larvae in chicken manure, and also administered as a pour on to prevent blow-fly strikes on sheep, but not on lactating animals producing milk for human consumption.

From the residue studies with cyromazine in sheep (see section 6.1.2) it may be concluded that residue levels of melamine in meat are maximally 1 % of total tissue radioactivity, about 80 % of which consists of cyromazine. Based on this information, it could be estimated that at the levels of the MRL for cyromazine (300 µg/kg of meat), the concentration of melamine in sheep meat will be 4 µg/kg at the most.

For the use of cyromazine as a plant protection product and as an insecticide in animal production, the MRL for the parent substance cyromazine does not consider the metabolite/breakdown melamine. Therefore the MRL values do not indicate directly what the residual level of melamine may be, but such levels can be estimated using two scenarios:

- Plant protection scenario: in the absence of specific data, an application rate of cyromazine on a range of crops was assumed at 75 to 225 g/ha as a foliar spray and up to 450 g/ha as a drench or drip. At an application rate of 450g/ha with a single application, a 10 % uptake rate, full metabolism to melamine, no subsequent fall in residues, and a cropping yield of 20 tonnes/ha (lettuce ca 20 tonnes, potatoes ca. 40 t/ha), a melamine residue of 1 mg/kg can be calculated. Data from the JMPR evaluation indicate that in celery, tomatoes and lettuce, melamine may represent up to 11-44 % of the total radioactive residue and cyromazine would represent 29-76 %. The highest MRL for cyromazine in plants is 10 mg/kg plant material (cabbage, spinach and mustard green). Based on the medians of the range (22 % = melamine and 50 % = cyromazine), an amount of melamine of 4.4 mg/kg plant

product can be estimated at this MRL. In mushrooms, the MRL for cyromazine is 7 mg/kg and melamine has been estimated to represent up to > 50 % of the total residue. The JMPR evaluation gives some indications that cyromazine could be used in forage plants (e.g. alfalfa, cotton (seed) and sudangrass hay) but it is not used in practice on such crops, so no melamine residue concentration have been estimated in animals from forages.

- Veterinary scenario: at the MRL for cyromazine, the maximum levels of melamine found in animal tissues may be estimated from experimental data to be 4 μg/kg. Meat with cyromazine levels higher than its MRL would be illegal for the consumers' market. In addition, cyromazine (and melamine) may be taken up from soils fertilized with manure from animals given cyromazine as a feed additive. However, no data is available to estimate the transfer level. Again, the data are not detailed enough to include this in the exposure assessment, but given the low concentrations that are estimated, these routes of exposure are unlikely to contribute significantly to human exposure.

#### 4.4.6. Background level from flame retardants

No specific information is available on the occurrence of melamine in food resulting from it use as a flame retardant but any such contamination would be included in the data for background levels. As melamine is not persistent or cumulative; its use as a flame retardant is not anticipated to be a major source in food and feed either currently or in the future.

### 4.5. Occurrence levels of cyanuric acid in food and feed

As previously highlighted in the introduction to the data collection chapter, no data on cyanuric acid occurrence were submitted by the European countries. The only source of analytical data on this substance is therefore the joint dataset submitted by CIAA, CEPE and Empac representing data collected by the industry during self-control of ingredients and products.

#### 4.5.1. Cyanuric acid occurrence data in food provided by industry

The collection of data on cyanuric acid occurrence in food measured by the industry includes 8059 samples. In analogy to the data on melamine occurrence, the cyanuric acid data were coded according to the food groups of the Concise Food Consumption Database (EFSA, 2008b) and the occurrence statistics were calculated for the different food groups. When values were reported for concentrated or dried food items, dilution factors have been applied. All the samples appeared to be reported as Upper Bound values, therefore only the Upper bound scenario is presented. Table 17 shows the occurrence statistics for cyanuric acid in food according to the database from the industry.

**Table 17:** Statistics on occurrence of cyanuric acid in food (mg/kg) in categories of the Concise Food Consumption database from the CIAA, CEPE and Empac dataset.

| Concise categories | Upper bound [1] (mg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | **n** | **min** | **mean** | **median** | **P95** | **Max** |
| 01A.Cereal-based mixed dishes | 3 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| 01B.Cereals & cereal products excl. cereal-based mixed dishes | 1299 | 0.01 | 0.07 | 0.05 | 0.2 | 5 |
| *01.Cereals & cereal products* | *1302* | *0.01* | *0.07* | *0.05* | *0.2* | *5* |
| *02.Sugar & sugar products including chocolate* | *612* | *0* | *0.05* | *0.05* | *0.11* | *0.81* |
| *03.Fats (vegetable and animal)* | *115* | *0.05* | *0.07* | *0.05* | *0.1* | *0.5* |
| 04A.Vegetable soups | 25 | 0.01 | 0.07 | 0.07 | 0.15 | 0.19 |
| 04B.Vegetables, nuts, pulses except vegetable soups | 710 | 0.01 | 0.16 | 0.05 | 0.5 | 5 |
| *04.Vegetables, nuts, pulses including carrots, tomato and leafy vegetables* | *735* | *0.01* | *0.15* | *0.05* | *0.5* | *5* |
| *05.Starchy roots or potatoes* | *42* | *0.01* | *0.06* | *0.05* | *0.17* | *0.26* |
| *06.Fruits* | *99* | *0.01* | *0.05* | *0.05* | *0.1* | *0.28* |
| 07A.Fruit and vegetable juices | 26 | 0.05 | 0.14 | 0.05 | 0.46 | 0.57 |
| 07B.Soft drinks with percentage of fruits lower than nectar, excl fruit juice | 1 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| *07.Fruit and vegetable juices, soft drinks and bottled water* | *27* | *0.05* | *0.13* | *0.05* | *0.46* | *0.57* |
| *08.Coffee, tea, cocoa (expressed as liquid)* | *139* | *0* | *0.01* | *0* | *0.05* | *0.05* |
| *09.Alcoholic beverages* | *3* | *0.05* | *0.05* | *0.05* | *0.05* | *0.05* |
| 10A.Meat and meat products and substitutes | 244 | 0.01 | 0.07 | 0.05 | 0.25 | 1.24 |
| 10B.Edible offal and offal products | 1 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| *10.Meat and meat products, offal* | *245* | *0.01* | *0.07* | *0.05* | *0.25* | *1.24* |
| 11A.Seafood and seafood products | 33 | 0.01 | 0.04 | 0.05 | 0.05 | 0.2 |
| 11B.Fish and fish products | 79 | 0.01 | 0.05 | 0.05 | 0.1 | 0.5 |
| *11.Fish and seafood* | *112* | *0.01* | *0.05* | *0.05* | *0.1* | *0.5* |
| *12.Eggs* | *209* | *0.01* | *0.02* | *0.01* | *0.05* | *0.2* |
| 13A.Milk and dairy-based drinks | 1878 | 0 | 0.03 | 0.01 | 0.06 | 0.82 |
| 13B.Dairy-based products | 757 | 0.01 | 0.03 | 0.01 | 0.2 | 0.5 |
| 13C.Cheese | 453 | 0.01 | 0.11 | 0.05 | 0.25 | 0.85 |
| *13.Milk and dairy-based products* | *3088* | *0* | *0.04* | *0.01* | *0.21* | *0.85* |
| 14A.Miscellaneous | 1021 | 0 | 0.05 | 0 | 0.3 | 1.43 |
| 14B.Food for special dietary uses | 273 | 0.01 | 0.02 | 0.01 | 0.05 | 0.12 |
| *14.Miscellaneous / Food for special dietary uses* | *1294* | *0* | *0.04* | *0.01* | *0.25* | *1.43* |
| *15.Tap water* | *17* | *0.01* | *0.04* | *0.05* | *0.05* | *0.05* |
| *Not classified* | *20* | *0.05* | *0.08* | *0.05* | *0.25* | *0.3* |
| | *8059* | *0* | *0.06* | *0.05* | *0.20* | *5* |

[1] A large majority of the samples appear to be reported as Upper Bound values (i.e. as their limit of detection); therefore the few hundred samples reported as not detected were also attributed the value of their limit of detection; n: number of samples; min: minimum value; P95: 95$^{th}$ percentile; max: maximum value.

Few samples with values near to 10 mg/kg were reported in the original database, before applying dilution factors. Similarly to what observed for melamine, these higher values correspond to samples of ferric pyrophosphate likely to have been subject to cross-contamination and are therefore included in the exposure assessment.

### 4.5.2. Cyanuric acid occurrence data in feed provided by industry

The CIAA, CEPE and Empac dataset contained 310 occurrence data for cyanuric acid in feed. No detailed grouping for feed samples was provided and, similarly to melamine, two groups were discriminated: in this case Cattle feed and Petfood.

One single cattle feed sample collected in late 2008, showing a value above 10 mg/kg feed, was considered adulterated and excluded from the dataset. The statistical descriptors for cyanuric acid occurrence are given in Table 18. It has to be noted that the samples of cattle feed are only 20 and all were below the limit of detection.

**Table 18:** Occurrence values for cyanuric acid (mg/kg) measured in feed categories.

| Feed Category | Upper bound (mg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | **n** | **min** | **mean** | **median** | **P95** | **max** |
| Cattle feed | 20 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Pet food | 289 | 0.05 | 0.25 | 0.05 | 1.42 | 4.78 |
| | *309* | *0.05* | *0.29* | *0.05* | *1.24* | *4.78* |

n: number of samples; min: minimum value; P95: 95$^{th}$ percentile; max: maximum value.

## 5. Exposure assessment in animals and humans

### 5.1. Exposure assessment for melamine in animals and humans, excluding infant formula

### 5.1.1. Exposure assessment for melamine in animals

5.1.1.1. Estimating melamine intake in feed by farm livestock

The exposure to melamine has been estimated in livestock based on three scenarios:

- melamine can be present as an impurity in urea-based commercial feed additive used in ruminants. The specification for one urea-based commercial product defines levels of melamine up to 50 mg/kg. A quantity of 30 g is used as feed additive per 100 kg animal, giving a daily intake of 15 µg/kg b.w. of melamine;

- cyromazine is used as a feed additive in poultry for the control of fly larvae in poultry manure. The specification for one feed additive product is 5 mg/kg feed and, assuming a 10 % breakdown of cyromazine to melamine, this would correspond to an exposure of 36 and 30 µg/kg b.w. per day based on 0.115 kg and 0.15 kg daily feed consumption in broilers (2.1 kg) and laying hens (1.9 kg), respectively;

- hypothetical melamine concentrations in mixed feed and premixes were selected based on the action level of 2.5 mg/kg with a lower level of 0.5 mg/kg and a higher level of 10 mg/kg to calculate a range of exposures in farm animals. These scenarios encompass the background data submitted by industry (Table 9).

According to 4.4.5, a melamine residue level of 1 mg/kg plant material coming from the breakdown of cyromazine can be estimated. However, because cyromazine is not used in forage crops, this route of exposure was not included in the exposure assessment.

Water, as a source of melamine has not been included as there are no indications that melamine occurs in drinking water for farm animals. Estimates of feed intake are based on typical feeding regimens within Europe that have been applied as the basis of previous EFSA exposure estimates in livestock.

Table 19 illustrates the predicted exposure of monogastric livestock to melamine given complete feed and according to the above assumptions. Overall, fish would be the least exposed to melamine (50 or 200 µg/kg b.w. per day, respectively, if feed contains 2.5 or 10 mg/kg melamine). Poultry (broilers) would be the most exposed to melamine (179 or 714 µg/kg b.w. respectively if feed contains 2.5 or 10 mg/kg melamine) and laying hens would be exposed to 151 or 605 µg/kg b.w respectively if feed contains 2.5 or 10 mg/kg melamine.

**Table 19:** Predicted exposure to melamine in monogastric livestock given a diet containing 0.5, 2.5 and 10 mg/kg melamine in feed.

| Species | Live weight (kg) | Consumption of total complete feed kg per day | Melamine exposure µg/kg b.w. per day | | |
|---|---|---|---|---|---|
| | | | 0.5 | 2.5 | 10 |
| Finishing pigs | 100 | 3.7 | 19 | 93 | 370 |
| Sows | 250 | 6.5 | 13 | 65 | 260 |
| Poultry (broilers) | 2.1 | 0.15 | 36 | 179 | 714 |
| Poultry (laying hens) | 1.9 | 0.115 | 30 | 151 | 605 |
| Fish | 4.5 | 0.09 | 10 | 50 | 200 |

Table 20 illustrates the predicted exposure of ruminants given compound feed according to the above assumptions. Growing cattle and suckler cows would be the least exposed to melamine (17 and 18 µg/kg b.w. per day, respectively if compound feed contained 2.5 mg/kg melamine, or 67 and 73 µg/kg b.w. per day respectively if compound feed contains 10 mg/kg melamine). Dairy goats and dairy cows would be the most exposed to melamine (42 and 40 µg/kg b.w. per day, respectively if compound feed contained 2.5 mg/kg melamine, or 169 and 160 µg/kg b.w. per day if compound feed contained 10 mg/kg melamine respectively).

**Table 20:** Predicted exposure to melamine in ruminants given compound feed containing 0.5, 2.5 and 10 mg/kg melamine. These calculations assume typical livestock feed and represent a conservative estimate situation.

| Species | Live weight (kg) | Consumption | | | |
|---|---|---|---|---|---|
| | | Compound feed kg per day | Melamine intake µg/kg b.w per day | | |
| | | | 0.5 | 2.5 | 10 |
| Dairy cow | 625 | 10 | 8 | 40 | 160 |
| Suckler cow | 550 | 4 | 4 | 18 | 73 |
| Growing cattle | 300 | 2 | 3 | 17 | 67 |
| Lactating ewe | 70 | 0.9 | 6 | 32 | 129 |
| Growing lamb | 20 | 0.3 | 8 | 38 | 150 |
| Dairy goats | 65 | 1.1 | 8 | 42 | 169 |

5.1.1.2. Estimation of melamine intake in pet food by pet animals

For cats and dogs the exposure is difficult to estimate due to the large variability in the composition of commercial diets. However, pet food is standardised with respect to the amount of protein that cats or dogs should eat on a daily basis. Standard text books indicate that dogs require 2 – 4 g protein/kg b.w. per day to maintain lean body mass, whereas cats require 5 – 8 g protein/kg b.w. per day. These protein levels in the pet food can be used as a standard to estimate the exposure to melamine in pets via feed from canned food and dried food with melamine contamination assumed to be 0.5, 2.5 and 10 mg/kg per kg canned or dried food. Based on information from industry on a range of products and assuming body weights of 3.5 kg for cats and 15 kg for dogs, the average amount of canned food and

dried food consumed, adjusted to the protein needs in cats and dogs, is 48 (range 31.5-97.5) g/kg b.w per day and 13.5 g/kg b.w. per day (8-25g/kg) respectively. The results of estimating melamine exposure for the range of consumption of canned food and dried food are illustrated in Table 21. Predicted exposure in cats and dogs is 120 (range 79-244) µg/kg b.w. per day for canned food and 34 (20-63) µg/kg b.w. per day for dried food containing 2.5 mg/kg melamine. At 10 mg/kg melamine, predicted exposure is 480 (320-980) µg /kg b.w. per day for canned food and 135 (80-250 µg/kg b.w. per day) for dried food.

**Table 21:** Predicted exposure to melamine in cats and dogs using canned food or dried food consumption patterns and contamination with melamine at 0.5, 2.5 and 10 mg /kg. These calculations assume the sole consumption of each product in cats and dogs and represent conservative estimates.

| **Melamine exposure µg/kg b.w per day Mean (range)** | | | | | |
|---|---|---|---|---|---|
| **Canned food[1*]** | | | **Dried Food[2*]** | | |
| 0.5 | 2.5 | 10 | 0.5 | 2.5 | 10 |
| 24 (16-49) | 120(79-244) | 480 (320-980) | 6.8(4-13) | 34(20-63) | 135 (80-250) |

[1] Canned food consumption per day is adjusted to protein consumption and is assumed to range between 31-97.5g/kg b.w. with an average of 48g/kg b.w.; [2] Consumption of dried food per day is adjusted to protein consumption and is assumed to range between 8 and 25 g/kg b.w. with an average of 13.5 g/kg b.w.*Protein consumption per day is assumed to be 8 and 4 g protein b.w. per day in cats and dogs, respectively, with average body weights of 3.5 kg and 15 kg respectively.

### 5.1.2. Exposure assessment in humans

5.1.2.1. Food consumption

The EFSA Concise European Food Consumption database was established by EFSA to support exposure assessments carried out in the EU. So far 19 countries have provided national data to EFSA for the database. To obtain comparable results, data were aggregated into 15 broad food groups, although some Member States provided data also for certain subgroups providing up to a total of 28 separate food class entries. The consumption figures for the food groups are linked to individual data on sex, age and body weight. The main statistics of the data are available on the EFSA website and contain mean consumption, median and standard deviation, as well as several low and high percentiles of consumption for the general population and for consumers only.

The concise database is intended to be used as a screening tool for exposure assessment as well as a first step towards generating a more comprehensive database. It allows assessment of the overall exposure of population groups to a wide variety of substances. Limitations arise from the broad food categories defined and from the different methodologies of data collection applied in different countries. The use of this database may be sufficient when the exposure calculation, based on conservative assumptions for concentrations, is below the level of concern. If this is not the case, further refinements might be necessary, particularly when defining sub-categories of interest and adjusting means using the appropriate sampling adjustment factor (SAF). A guidance document for the use of the data has been published on the EFSA website (see Annex 3 to EFSA, 2008b)

For calculating melamine and cyanuric acid exposures, data at the individual level were accessed in the database. In this way, the 95$^{th}$ percentile exposure in particular can be calculated more accurately than by using the method described in the guidance document. It is also possible to use the individual weight as recorded rather than a standard weight of 60 kg.

5.1.2.2. Food consumption for infants and young children

Infants and young children are often more highly exposed than adults to chemicals in food when considering the exposure in relation to body weight. According to the Institute of Medicine (IOM) average breast milk consumption is about 750-800 g per day (range: 450-1,200 g per day) for the first 4-5 months of life (IOM, 1991). Infant birth weight and nursing frequency have been shown to influence the rate of intake (IOM, 1991). The German DONALD study looked at consumption of infant formula and found that a 3 months old child weighing on average 6.1 kg consumed a mean of 780 mL per day with a 95$^{th}$ percentile consumption of 1,060 mL per day (Kersting et al., 1998). Taking into account both studies, a rounded mean value of 800 g per day and a rounded high value of 1100 g per day were used for calculating melamine and cyanuric acid exposure from milk formula.

5.1.2.3. Exposure in adults based on submitted data

For this opinion, melamine dietary exposure was calculated separately for each country for the whole population using consumption data recorded at the individual level. The mean and P95 of the upper bound of each food category from the CIAA, CEPE and Empac joint dataset (Table 5) were used as occurrence figures, combined with all the individual consumption data from the Concise food consumption database for the calculation of the exposure. The cumulative exposure from all food categories was separately calculated for each individual. The exposures calculated for the subjects in each country were then aggregated to calculate the exposure statistics for that country. As noted in section 4.2, the occurrence data submitted by European countries were not used in the exposure assessment because they were the results of targeted sampling not representative of foods on the EU market. Exposure of adults to melamine calculated in European countries using the mean and P95 occurrence of each food category is presented in Table 22. For the worst case scenario based on upper bound P95 occurrence data, the P95 exposure estimates are below 11 µg/kg b.w. per day for all countries. Because the exposure estimates were very much lower than the TDI (see section 7.2) a probabilistic exposure assessment was not considered necessary.

**Table 22:** Total dietary exposure to melamine (µg/kg b.w. per day) for average (mean) and 95th percentile consumers (P95) across a number of subjects (N) in European countries (MS) using the mean and the P95 of the upper bound (UB) melamine concentrations from the CIAA, CEPE and Empac joint dataset (Table 5)

| MS | N | Exposure (µg/kg b.w. per day) based on mean occurrence (UB) | | Exposure (µg/kg b.w. per day) based on P95 occurrence (UB) | |
|---|---|---|---|---|---|
| | | MEAN exposure | P95 of exposure | MEAN exposure | P95 of exposure |
| AT | 2123 | 1.79 | 3.44 | 5.11 | 10.33 |
| BE | 1723 | 1.42 | 2.43 | 3.97 | 6.89 |
| BG | 853 | 1.22 | 2.32 | 3.76 | 6.99 |
| CZ | 1751 | 1.4 | 2.35 | 4.12 | 6.81 |
| DE | 3550 | 1.63 | 2.8 | 4.83 | 8.3 |
| DK | 3150 | 2.04 | 3.57 | 6.16 | 10.58 |
| EE | 2010 | 1.56 | 2.87 | 4.29 | 7.86 |
| FI | 2007 | 1.92 | 3.05 | 5.57 | 9.04 |
| FR | 1195 | 1.59 | 2.5 | 4.7 | 7.36 |
| GB | 1724 | 1.27 | 2.17 | 3.6 | 6.3 |
| HU | 927 | 1.36 | 2.05 | 4.05 | 6.21 |
| IE | 1373 | 1.79 | 3.04 | 5.24 | 8.96 |
| IS | 1075 | 2.16 | 3.92 | 5.63 | 9.95 |
| IT | 1544 | 1.6 | 2.46 | 4.98 | 7.82 |
| NL | 4285 | 1.58 | 2.53 | 4.93 | 7.97 |
| NO | 2321 | 1.69 | 3.07 | 4.61 | 8 |
| PL | 2692 | 1.88 | 3.37 | 5.38 | 9.78 |
| SE | 1088 | 1.78 | 2.98 | 5.25 | 8.68 |
| SK | 2208 | 1.09 | 2.57 | 2.66 | 6.45 |
| minimum | | 1.09 | 2.05 | 2.66 | 6.21 |
| median | | 1.6 | 2.8 | 4.83 | 7.97 |
| maximum | | 2.16 | 3.92 | 6.16 | 10.58 |

AT: Austria; BE: Belgium; BG: Bulgaria; CZ: Czech Republic; DE: Germany; DK: Denmark; EE: Estonia; FI: Finland; FR: France; GB: Great Britain; HU: Hungary; IE: Ireland; IS: Iceland; IT: Italy; NL: The Netherlands; NO: Norway; PL: Poland; SE: Sweden; SK: Slovak Republic; N: number of samples; UP: upper bound; MS: Member States; P95: 95th percentile.

The variation in exposure between countries is influenced by different consumption patterns only, since melamine concentrations in food categories were considered common at a European level. The contribution of each food category to melamine exposure was calculated from both the mean and P95 upper bound melamine concentrations expressed in mg/kg from the CIAA, CEPE and Empac dataset using consumption data recorded at the individual level.

The contribution of each food category, expressed in µg/kg b.w. per day, to the overall melamine exposure is described using the mean values and the P95 of melamine occurrence data in Table 23 and Table 24 respectively.

**Table 23:** Statistics on exposure to melamine (µg/kg b.w. per day) based on mean UB occurrence calculated across the European countries for which consumption data at individual level are present in the Concise food consumption database using the mean of the upper (UB) bound concentrations from the CIAA, CEPE and Empac joint dataset (Table 5) and mean and P95 of consumption.

| Food category | Exposure statistics across countries based on MEAN of consumption µg/kg b.w. per day | | | Exposure statistics across countries based on P95 of consumption µg/kg b.w. per day | | |
|---|---|---|---|---|---|---|
| | min | median | max | min | median | max |
| 01.Cereals & cereal products | 0.14 | 0.23 | 0.28 | 0.27 | 0.44 | 0.58 |
| 02.Sugar & sugar products including chocolate | 0.01 | 0.02 | 0.04 | 0.03 | 0.06 | 0.1 |
| 03.Fats (vegetable and animal) | 0.01 | 0.03 | 0.05 | 0.04 | 0.07 | 0.12 |
| 04.Vegetables, nuts, pulses including carrots, tomato and leafy vegetables | 0.2 | 0.24 | 0.35 | 0.46 | 0.57 | 0.91 |
| 05.Starchy roots or potatoes | 0.04 | 0.09 | 0.26 | 0.12 | 0.22 | 0.62 |
| 06.Fruits | 0.05 | 0.09 | 0.21 | 0.21 | 0.27 | 0.62 |
| 07.Fruit and vegetable juices, soft drinks and bottled water | 0.12 | 0.17 | 0.29 | 0.41 | 0.56 | 1.04 |
| 08.Coffee, tea, cocoa (expressed as liquid) | 0.01 | 0.05 | 0.09 | 0.04 | 0.13 | 0.19 |
| 09.Alcoholic beverages | 0.09 | 0.1 | 0.1 | 0.48 | 0.55 | 0.62 |
| 10.Meat and meat products, offal | 0.1 | 0.13 | 0.13 | 0.23 | 0.23 | 0.37 |
| 11.Fish and seafood | 0.02 | 0.02 | 0.02 | 0.07 | 0.08 | 0.12 |
| 12.Eggs | 0 | 0.01 | 0.01 | 0.02 | 0.02 | 0.06 |
| 13.Milk and dairy-based products | 0.2 | 0.25 | 0.26 | 0.54 | 0.58 | 0.64 |
| 14.Miscellaneous / Food for special dietary uses | 0 | 0 | 0.01 | 0.01 | 0.01 | 0.05 |
| 15.Tap water | 0 | 0.21 | 0.65 | 0 | 0.79 | 1.75 |

b.w.: body weight; UB: Upper bound, min: minimum value; P95: 95$^{th}$ percentile; max: maximum value

**Table 24:** Statistics on exposure to melamine (µg/kg b.w. per day) based on P95 UB occurrence calculated across the European countries for which consumption data at individual level are present in the Concise food consumption database using the P95 of the upper (UB) bound concentrations from the CIAA, CEPE and Empac joint dataset (Table 5) and the mean and P95 of consumption.

| Food category | Exposure statistics across countries based on MEAN of consumption µg/kg b.w. per day | | | Exposure statistics across countries based on P95 of consumption µg/kg b.w. per day | | |
|---|---|---|---|---|---|---|
| | min | median | max | min | median | max |
| 01.Cereals & cereal products | 0.51 | 0.82 | 1.02 | 0.97 | 1.59 | 2.09 |
| 02.Sugar & sugar products including chocolate | 0.02 | 0.03 | 0.05 | 0.04 | 0.08 | 0.12 |
| 03.Fats (vegetable and animal) | 0.02 | 0.05 | 0.09 | 0.07 | 0.12 | 0.2 |
| 04.Vegetables, nuts, pulses including carrots, tomato and leafy vegetables | 0.84 | 1 | 1.47 | 1.93 | 2.36 | 3.78 |
| 05.Starchy roots or potatoes | 0.04 | 0.09 | 0.25 | 0.12 | 0.21 | 0.6 |
| 06.Fruits | 0.06 | 0.1 | 0.25 | 0.25 | 0.32 | 0.74 |
| 07.Fruit and vegetable juices, soft drinks and bottled water | 0.12 | 0.17 | 0.29 | 0.4 | 0.55 | 1.03 |
| 08.Coffee, tea, cocoa (expressed as liquid) | 0.09 | 0.39 | 0.61 | 0.26 | 0.9 | 1.35 |
| 09.Alcoholic beverages | 0.09 | 0.1 | 0.1 | 0.48 | 0.55 | 0.62 |
| 10.Meat and meat products, offal | 0.2 | 0.27 | 0.29 | 0.48 | 0.49 | 0.79 |
| 11.Fish and seafood | 0.03 | 0.04 | 0.05 | 0.13 | 0.17 | 0.23 |
| 12.Eggs | 0.01 | 0.03 | 0.04 | 0.06 | 0.08 | 0.21 |
| 13.Milk and dairy based products | 0.89 | 1.09 | 1.17 | 2.39 | 2.58 | 2.83 |
| 14.Miscellaneous / Food for special dietary uses | 0 | 0.01 | 0.03 | 0.01 | 0.02 | 0.11 |
| 15.Tap water | 0 | 0.59 | 1.8 | 0 | 2.17 | 4.81 |

b.w.: body weight; UB: Upper bound; min: minimum value; P95: 95$^{th}$ percentile; max: maximum value

The exposure is mainly driven by the consumption figure for the respective food items, milk, water, vegetables and cereals, rather than by the presence of melamine. These values may be considered conservative, because the occurrence data are in most cases upper bound values for not detected samples, as discussed in chapter 4.2.

5.1.2.4. Potential exposure of adults to melamine resulting from use of cyromazine

Melamine residues in sheep resulting from cyromazine at the maximum residue level (300 µg/kg of meat) would reach a maximum of 4 µg/kg in meat. This would result in an exposure to melamine below 0.020 µg/kg b.w. per day, considering meat consumption of 300 g in a 60 kg adult. Following the use of cyromazine in poultry feed to control diptera larvae, up to 10 % of the given dose might be converted to melamine. If the entire melamine fraction would be disposed in muscle tissue, this would result in a residue level of 30-36 µg/kg of meat of layers and broilers, respectively, and an exposure of below 0.020 µg/kg b.w. per day considering a meat consumption of 300 g poultry meat in a 60 kg adult. Ammeline was tentatively identified as a minor metabolite in eggs of layers exposed to cyromazine, but the concentrations remained below the limit of detection and hence residues in eggs are not expected to contribute to human exposure.

The transfer of melamine to eggs has been estimated to be 0.16, 0.47, 0.84 and 1.48 mg/kg egg in laying hens, given an experimental feed spiked with 0, 5, 25, 50 or 100 mg of melamine per kg over a period of 15 days 0, 5, 25, 50 and 100 mg/kg feed treated groups (Chen et al., 2010) (see section 6.1.1.4.2 for details). Transfer rates were calculated to vary between 0.015 and 0.032. Comparing these findings with the 10 mg/kg melamine in feed, residue levels in eggs are expected to range

between 0.16 and 0.47 mg/kg egg and, given a daily consumption of eggs in adults of 100 g, this would result in an exposure of 16 – 47 µg/per person or 260 –780 µg/ kg b.w. These experimental data are in the same order of magnitude as the exposure levels calculated on the basis of the Concise food consumption database using the P95 of consumption and P95 of occurrence (0.21µg/kg b.w.) (Table 24).

Overall, applying a scenario for animal exposure with a maximum contamination at the level of 10 mg/kg of melamine in the feed of laying hens, a maximum transfer rate into eggs of 3.2 % as estimated from the experimental studies, together with a maximum consumption of 100 g of eggs per day by an adult person, the daily intake would be below 1 µg/kg b.w.

#### 5.1.2.5. Contributions of food contact materials to melamine dietary exposure

##### 5.1.2.5.1. Rough exposure assessment from all food contact materials

All possible sources of exposure to melamine from food contact materials need to be considered in the exposure assessment:

- tableware made with melaware;
- canned foods when the can coating contains melamine;
- adhesives and other miscellaneous food packaging applications.

Since the effects of melamine can potentially arise from acute exposure, the exposure assessment should be based on conservative assessment of acute levels of consumption (high percentile of consumption within one day) combined with conservative assessment of migration (either current specific migration limit or highest observed levels of migration in products currently on the market).

For a first rough exposure assessment, the same scenario of food consumption used in the opinion on bisphenol A (BPA) was considered (EFSA, 2006a). In fact, the patterns of use of tablewares made with melaware are likely to be similar to the patterns of use of tablewares made with polycarbonate. In the opinion on bisphenol A it was considered that all foods and beverages could be in contact with plastic tablewares (with the exception of infant formula that would be in contact with baby bottles). In the same opinion, in order to assess exposure to BPA from epoxy resins of cans, it was assumed that all commercial foods and beverages, including powdered infant formula, could be canned. Both sources of exposure were added.

In the case of melamine, exposure may also occur from contact of food with paper and board or adhesive. However, since food can be in contact either with can or with paper and board and since the highest potential migration may occur in cans, the first conservative exposure scenario considered was that of contact of all foods and beverages with cans.

The aim of the first conservative exposure scenario is to identify the population group that may be more exposed and the main sources of exposure so as to focus further refined exposure assessments if needed. As for BPA three age groups were considered (see Table 25).

**Table 25:** Identification of age groups with the highest theoretical exposure to melamine from food contact materials[1]

| Age group | Consumption of foods and beverages (kg)[(1)] | Body weight (kg)[(1)] | Consumption of foods and beverages[(1)] (g/kg b.w.) | Melamine exposure (µg/kg b.w.) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Melaware[2] (SML) | Cans[3] (highest migration) | Melaware[4] (highest migration) | Melaware and cans[5] (highest migration |
| Infant aged 6 months | 0.407 plus 0.125 of powdered infant formula | 7.8 | 52 plus 16 of powdered infant formula | 1500 (750 % ) | 34 (17 %) | 260 (130 %) | 294 (147 %) |
| Child aged 1.5 years | 2 | 11 | 182 | 5500 (2750 %) | 92 (46 %) | 910 (455 %) | 1002 (501 %) |
| Adult | 3 | 60 | 50 | 1500 (750 %) | 25 (13 %) | 250 (125 %) | 275 (138 %) |

[1]The three scenarios considered were taken from EFSA (2006a). The consumption of infant formula was not considered to estimate exposure to melamine from tableware since melaware is not used in baby bottles. Values considered for the 6 month infant are the 95th percentile of consumption of commercial baby foods and drinks and of powdered infant formula observed in 6 month infants in the DONALD study (Kersting et al., 1998). The body weight considered for the child aged 1.5 years consuming daily 2 kg of commercial foods (2/3 of beverages, 1/3 of solid foods) is that reported in CEC (1993). For adults the daily consumption of 1 kg of solid foods and 2 kg of beverages were considered. [2] Exposure from melaware at SML 30 mg/kg food; [3] Exposure from cans at maximum observed migration (0.5 mg/kg); [4] Exposure from melaware at highest migration level observed (5 mg/kg); [5] Exposure from melaware and cans at maximum observed migration level (5 and 0.5 mg/kg respectively). b.w.: body weight; SML: specific migration limit.

It appears from Table 25 that the maximum migration limit of 30 mg/kg melamine from tableware leads to a theoretical exposure of at least 1500 µg/kg b.w (more than 750 % of the TDI of 0.2 mg/kg b.w. per day, see section 6.6.1) in all ages considered. When considering conservative estimates of high migration levels from can coatings and from melaware, the cumulative theoretical exposure is higher than the TDI for the three age groups considered. The cumulative theoretical exposure is about 3 times higher in a child aged 1.5 years compared with that of an infant aged 6 month and of an adult, which are similar. Refinement of exposure should therefore focus primarily on small children. The highest potential source of exposure is melaware, but canned foods could also be responsible for a significant exposure, with the theoretical exposure reaching 46 % of the TDI in small children.

5.1.2.5.2. Refined exposure assessment from melaware in small children

Exposure to melamine from melaware in children was estimated by combining estimated migration levels with food consumption data gathered at EFSA through the EFSA art. 36 project: "Individual food consumption data and exposure assessment studies for children" (acronym EXPOCHI). Within this project, which started in the end of 2008, children food consumption data for 13 different Member States has been provided to EFSA at the individual level. Basic information concerning the food consumption databases included in the assessment are reported in Table 26. Only children from 1 to 2 and 3 to 6 years old were considered in the analysis. This selection caused the exclusion of all subjects from Cyprus since they are all between 11and 14 years of age. All food descriptors reported in the food consumption databases are currently organised into 43 food groups. In order to link melamine migration levels to the food descriptors present in the databases, each of the 43 food groups were classified according to a migration class as dry, fatty, aqueous or acidic (Table 27). In the case that the description of the food group could cover two or more food types, for example aqueous and acidic, then the food type with the highest migration value was allocated - in this example being acidic. Literature data were used to estimate typical and high melamine migration levels for each of the above mentioned four migration classes, as described in section 4.4.4.1.1. and summarised in Table 28. Exposure to melamine from melaware in children was assessed under two scenarios, called A and B.

Both scenarios are aimed at assessing acute exposure (within one day). In both scenarios, the melaware articles considered had a high migration potential.

Under scenario A, using typical migration levels, it was assumed that all foods and beverages consumed in one day came into contact with the melaware articles under normal time and temperature conditions. Exposure was obtained by summing up, within each day, the exposure from all food groups.

High melamine migration levels were used under scenario B. It was therefore assumed that any food item could come into contact with a melaware article under severe time and temperature contact conditions. In this case, daily exposure was not assessed by summing up the contributions from all food items but considering only the food item leading to the highest exposure values within one day. The rest of the food items eaten that day was therefore assumed not to be prepared and served in contact with melaware.

Melamine exposure levels per country, age class and scenario are reported in Table 29.

Total exposure in one day from all food groups considering typical migration levels (scenario A) ranged from 30 µg/kg b.w. to 80 µg/kg b.w. at the mean, and from 50 µg/kg b.w. to 120 µg/kg b.w. at the 95$^{th}$ percentile.

Highest exposure in one day from one food item considering high migration levels (scenario B) ranged from 40 µg/kg b.w. to 110 µg/kg b.w. at the mean, and from 70 µg/kg b.w. to.230 µg/kg b.w. at the 95$^{th}$ percentile.

**Table 26:** Basic information concerning the food consumption databases included in the EXPOCHI project

| Country | Year of survey | Represen-tativity | Number of subjects | Ages (years) | Number of survey days per subject | Dietary survey method | Reference |
|---|---|---|---|---|---|---|---|
| Belgium | 2002-2003 | Regional | 661 | 2-6 | 3 | Dietary record | Huybrechts et al., 2008 |
| Cyprus | 2002-2006 | National | 268 | 11-14 | 3 | Dietary record | www.childhealth.ac.cy |
| Czech Republic | 2003-2004 | National | 602 | 4-14 | 2 | 24-h recall | Ruprich et al., 2006 |
| Denmark | 2000-2002 | National | 610 | 4-10 | 7 | Dietary record | Lyhne et al., 2005 |
| Finland-DIPP | 2005 | Regional | 1,500 | 1, 3, 6 | 3 | Dietary record | Räsänen et al., 2006 |
| Finland-STRIP | 2000 | Regional | 250 | 7-8 | 4 | Dietary record | Simell et al., 2009 |
| France | 2005-2007 | Regional | 574 | 3-10 | 7 | Dietary record | AFSSA, 2009 |
| Germany | 2006 | Regional | 303 | 1-10 | 3 | Dietary record | Kroke et al., 2004 |
| Germany | 2007 | Regional | 311 | 1-10 | 3 | Dietary record | |
| Germany | 2008 | National | 307 | 1-10 | 3 | Dietary record | |
| Greece | 2004-2005 | Regional | 795 | 4-6 | 3 | Dietary record | Linardakis et al., 2008 |
| Italy | 2005-2006 | National | 252 | 1-10 | 3 | Dietary record | Leclercq et al., 2009 |
| Netherlands | 2005-2006 | National | 1,279 | 2-6 | 2 | Dietary record | Ocké et al., 2008 |
| Spain-Basque | 2004-2005 | Regional | 760 | 4-14 | 2 | 24-h recall | Larrañaga et al., 2006 |
| Spain-enKid | 1998-2000 | National | 382 | 1-14 | 2 | 24-h recall | Serra-Majem et al., 2001 |
| Sweden | 2003 | National | 2,298 | 3-13 | 4 | Dietary record | Enghardt-Barbieri et al., 2006 |

**Table 27:** Food groups per melamine migration class.

| | Food group | Melamine migration class |
|---|---|---|
| 1 | Composed foods – Cereal-based mixed dishes and cereal-based desserts | Aqueous |
| 2 | Vegetables excl. dried vegetables | Aqueous |
| 3 | Nuts and seeds | Dry |
| 4 | Coffee and tea in concentrated and in powdered form | Aqueous |
| 5 | Chocolate and chocolate products | Fatty |
| 6 | Fruit excl. dried fruit | Acidic |
| 7 | Dried fruit | Acidic |
| 8 | Fresh and dried herbs, spices, seasonings and condiments | Aqueous |
| 9 | Food supplements | Aqueous |
| 10 | Waters | Aqueous |
| 11 | Sugar, sweeteners and sugar products (e.g. sugar based confectionery, chewing gum and decorations) | Aqueous |
| 12 | Fats, oils and fat emulsions (also e.g. rice milk (no soy milk)) | Aqueous |
| 13 | Composed foods: meat-based mixed dishes | Aqueous |
| 14 | Composed foods: fish-based mixed dishes | Aqueous |
| 15 | Dried vegetables | Aqueous |
| 16 | Pulses and legumes | Aqueous |
| 17 | Soy milk and soy-based dessert | Aqueous |
| 18 | Milk and dairy drinks | Aqueous |
| 19 | Cheese | Fatty |
| 20 | Dairy-based products | Aqueous |
| 21 | Salt | Dry |
| 22 | Fish | Aqueous |
| 23 | Molluscs | Aqueous |
| 24 | Cephalopods | Aqueous |
| 25 | Crustaceans | Aqueous |
| 26 | Other seafood (echinoderms) | Aqueous |
| 27 | Beer and malt beverages | Aqueous |
| 28 | Wine and substitutes | Aqueous |
| 29 | Other alcoholic beverages | Aqueous |
| 30 | Fruit juices and nectars. | Acidic |
| 31 | Vegetable juices and nectars | Acidic |
| 32 | Soft drinks and edible ices | Acidic |
| 33 | Cereals and cereal products (no cereal based desserts or cereal based mixed dishes) | Aqueous |
| 34 | Other food for special dietary uses | Aqueous |
| 35 | Infant formulae, follow up formulae, food for young children and infant formulae and follow up formulae for medical purposes | Dry |
| 36 | Miscellaneous foods/products | Aqueous |
| 37 | Liver and kidney | Aqueous |
| 38 | Offals except liver and kidney | Aqueous |
| 39 | Types of vegetarian substitutes for meat and fish | Aqueous |
| 40 | Fresh meat | Aqueous |
| 41 | Processed meat | Aqueous |
| 42 | Coffee and tea in liquid form | Aqueous |
| 43 | Eggs | Aqueous |

**Table 28:** Estimated melamine migration levels per migration class.

| Melamine migration class | Melamine migration levels (mg/kg) | |
|---|---|---|
| | Typical | High |
| Dry | 0.05 | 0.05 |
| Aqueous | 0.6 | 3.0 |
| Fatty | 0.2 | 1.0 |
| Acidic | 1.0 | 5.0 |

**Table 29:** Melamine exposure levels per country, age class and scenario.

| Country | Age class (years) | Total number of survey days | Mean body weight (kg) | Mean total food consumption (g/kg b.w.) | Melamine exposure (µg/kg b.w.) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Scenario A[1] | | Scenario B[2] | |
| | | | | | Mean | P95 | Mean | P95 |
| Belgium | 1 to 2 | 126 | 14.0 | 113.0 | 80 | 120 | 70 | 120 |
| Belgium | 3 to 6 | 1833 | 17.9 | 92.1 | 60 | 100 | 60 | 90 |
| Czech Republic | 3 to 6 | 364 | 21.4 | 87.4 | 60 | 100 | 90 | 210 |
| Germany (2006) | 1 to 2 | 207 | 14.2 | 95.8 | 60 | 100 | 100 | 230 |
| Germany (2006) | 3 to 6 | 249 | 19.4 | 78.5 | 50 | 80 | 80 | 170 |
| Germany (2007) | 1 to 2 | 183 | 14.1 | 94.3 | 60 | 90 | 90 | 190 |
| Germany (2007) | 3 to 6 | 282 | 19.4 | 77.8 | 50 | 80 | 80 | 160 |
| Germany (2008) | 1 to 2 | 165 | 14.6 | 99.4 | 60 | 120 | 110 | 240 |
| Germany (2008) | 3 to 6 | 270 | 19.7 | 78.5 | 50 | 80 | 80 | 150 |
| Denmark | 3 to 6 | 1753 | 21.8 | 85.9 | 50 | 80 | 50 | 100 |
| Spain (NUT_INK05) | 3 to 6 | 312 | 21.9 | 88.4 | 50 | 80 | 50 | 80 |
| Spain (enKid) | 1 to 2 | 66 | 15.8 | 103.5 | 60 | 90 | 110 | 210 |
| Spain (enKid) | 3 to 6 | 134 | 22.2 | 77.1 | 50 | 80 | 80 | 150 |
| Finland (DIPP) | 1 to 2 | 1364 | 15.1 | 105.5 | 70 | 100 | 50 | 90 |
| Finland (DIPP) | 3 to 6 | 1409 | 22.3 | 80.5 | 50 | 70 | 40 | 80 |
| France | 1 to 2 | 238 | 14.5 | 92.2 | 50 | 90 | 80 | 160 |
| France | 3 to 6 | 1437 | 19.4 | 78.7 | 50 | 80 | 70 | 160 |
| Cyprus | 3 to 6 | 2383 | 22.4 | 51.2 | 30 | 50 | 40 | 70 |
| Italy | 1 to 2 | 108 | 15.2 | 95.5 | 60 | 90 | 90 | 170 |
| Italy | 3 to 6 | 231 | 21.9 | 78.2 | 50 | 80 | 70 | 140 |
| Netherlands | 1 to 2 | 1280 | 15.4 | 98.1 | 70 | 100 | 60 | 120 |
| Netherlands | 3 to 6 | 1278 | 21.3 | 77.8 | 50 | 80 | 50 | 100 |
| Poland | 1 to 2 | 94 | 13.3 | 117.2 | 70 | 110 | 60 | 80 |
| Poland | 3 to 6 | 144 | 19.3 | 94.2 | 60 | 80 | 40 | 80 |
| Sweden | 3 to 6 | 2358 | 18.2 | 87.2 | 60 | 90 | 60 | 100 |

[1]Total exposure in one day from all food groups, considering normal migration levels; [2]Highest exposure in one day from one food group, considering maximum migration levels. b.w. body weight; P95: 95$^{th}$ percentile.

## 5.2. Exposure assessment for cyanurate in animals and adult humans

### 5.2.1. Exposure assessment for cyanurate in animals

Cyanurate is not given intentionally to animals, but may occur as an impurity in urea-based commercial feed additives for ruminants such as biuret, and has been found in pet food likely as a contaminant of scrap melamine, which is indicative for a non-authorized use of melamine. The specification for one urea-based commercial product defines levels of cyanurate up to 200 mg/kg. Assuming a quantity of 30 g is used as feed additive per 100 kg animal, giving a daily intake of 60 µg/kg b.w. per day of cyanurate. Occurrence of cyanuric acid has also been measured in cattle feed and values were all at or below the limit of detection of 1 mg/kg. In milk and dairy products and meat and meat products, values for cyanuric acid were below or close to 1 mg/kg (maximum value 1.43 mg/kg in meat and meat products - Table 17).

### 5.2.2. Exposure assessment for cyanurate in adult humans

The cyanuric acid dietary exposure was calculated separately for each country for the whole population using consumption data recorded at the individual level, as in the case of melamine. The mean and P95 of the upper bound of each food category from the CIAA, CEPE and Empac joint dataset (Table 5) were used as occurrence figures, combined with all the individual consumption data from the Concise food consumption database for the calculation of the exposure. The cumulative exposure from all food categories was separately calculated for each individual. The exposures calculated for the subjects in each country were then aggregated to calculate the exposure statistics for that country.Exposure of adults to cyanuric acid calculated in European countries using the mean and P95 occurrence of each food category is presented in Table 30. For the worst case scenario based on upper bound P95 occurrence data the P95 exposure estimates are below 16 µg/kg b.w. per day for all countries.

**Table 30:** Total dietary exposure to cyanuric acid (µg/kg b.w. per day) for average (mean) and 95th percentile consumers (P95) across a number of subjects (N) in European countries (MS) using the mean and the P95 of the upper bound (UB) cyanuric acid concentrations from the CIAA, CEPE and Empac joint dataset (Table 10).

| MS | N | Exposure (µg/kg b.w. per day) based on mean occurrence (UB) | | Exposure (µg/kg b.w. per day) based on P95 occurrence (UB) | |
|---|---|---|---|---|---|
| | | MEAN exposure | P95 of exposure | MEAN exposure | P95 of exposure |
| AT | 2123 | 1.98 | 3.88 | 5.11 | 10.53 |
| BE | 1723 | 1.63 | 2.77 | 4.38 | 7.53 |
| BG | 853 | 1.4 | 2.66 | 3.96 | 7.43 |
| CZ | 1751 | 1.49 | 2.57 | 4.08 | 6.9 |
| DE | 3550 | 1.9 | 3.4 | 5.54 | 10.13 |
| DK | 3150 | 2.08 | 3.54 | 5.12 | 8.4 |
| EE | 2010 | 2.01 | 3.76 | 6.39 | 11.91 |
| FI | 2007 | 2.1 | 3.5 | 5.91 | 10.14 |
| FR | 1195 | 1.73 | 2.65 | 4.66 | 7.18 |
| GB | 1724 | 1.36 | 2.33 | 3.45 | 5.78 |
| HU | 927 | 1.52 | 2.35 | 4.36 | 6.92 |
| IE | 1373 | 2.11 | 3.77 | 6.03 | 10.54 |
| IS | 1075 | 2.66 | 5.67 | 7.19 | 15.9 |
| IT | 1544 | 1.75 | 2.7 | 4.75 | 7.33 |
| NL | 4285 | 1.73 | 2.77 | 4.92 | 7.9 |
| NO | 2321 | 1.79 | 3.29 | 4.28 | 7.71 |
| PL | 2692 | 2.13 | 3.92 | 6.21 | 11.31 |
| SE | 1088 | 1.9 | 3.14 | 5.45 | 8.64 |
| SK | 2208 | 1.22 | 2.89 | 3.08 | 6.96 |
| minimum | | 1.22 | 2.33 | 3.08 | 5.78 |
| median | | 1.79 | 3.14 | 4.92 | 7.9 |
| maximum | | 2.66 | 5.67 | 7.19 | 15.9 |

AT: Austria; BE: Belgium; BG: Bulgaria; CZ: Czech Republic; DE: Germany; DK: Denmark; EE: Estonia; FI: Finland; FR: France; GB: Great Britain; HU: Hungary; IE: Ireland; IS: Iceland; IT: Italy; NL: The Netherlands; NO: Norway; PL: Poland; SE: Sweden; SK: Slovak Republic; MS: member states; N: number of subjects; UB: upper bound; P95: 95th percentile.

The variation in exposure between countries is influenced by different consumption patterns only, since cyanuric acid concentrations in food categories were considered common at a European level.

5.2.2.1. Contributions of different food groups to cyanuric acid exposure

The contribution of each food category to cyanuric acid exposure was calculated from both the mean and P95 upper bound cyanuric acid concentrations expressed in mg/kg from the CIAA, CEPE and Empac dataset (Table 10) using consumption data recorded at the individual level. The contribution of each food category, expressed in µg/kg b.w. per day, to the overall cyanuric acid exposure is described using the mean values and the P95 of cyanuric acid occurrence from the data in Table 17 is given in Tables 31 and Table 32, respectively.

**Table 31:** Statistics on dietary exposure to cyanuric acid (µg/kg b.w. per day) calculated across the European countries for which consumption data at individual level are present in the Concise food consumption database using the mean of the upper (UB) bound concentrations from the CIAA, CEPE and Empac joint dataset and the mean and P95 of consumption.

| Food category | Exposure statistics across countries based on MEAN of consumption µg/kg b.w. per day | | | Exposure statistics across countries based on P95 of consumption µg/kg b.w. per day | | |
|---|---|---|---|---|---|---|
| | min | median | max | min | median | max |
| 01.Cereals & cereal products | 0.15 | 0.24 | 0.29 | 0.28 | 0.46 | 0.6 |
| 02.Sugar & sugar products including chocolate | 0.01 | 0.03 | 0.05 | 0.04 | 0.07 | 0.11 |
| 03.Fats (vegetable and animal) | 0.01 | 0.03 | 0.06 | 0.04 | 0.08 | 0.14 |
| 04.Vegetables, nuts, pulses including carrots, tomato and leafy vegetables | 0.26 | 0.3 | 0.45 | 0.59 | 0.72 | 1.15 |
| 05.Starchy roots or potatoes | 0.05 | 0.1 | 0.27 | 0.13 | 0.23 | 0.66 |
| 06.Fruits | 0.05 | 0.09 | 0.22 | 0.22 | 0.29 | 0.67 |
| 07.Fruit and vegetable juices, soft drinks and bottled water | 0.33 | 0.44 | 0.77 | 1.07 | 1.48 | 2.73 |
| 08.Coffee, tea, cocoa (expressed as liquid) | 0.02 | 0.07 | 0.11 | 0.05 | 0.16 | 0.24 |
| 09.Alcoholic beverages | 0.09 | 0.1 | 0.1 | 0.48 | 0.55 | 0.62 |
| 10.Meat and meat products, offal | 0.11 | 0.15 | 0.16 | 0.27 | 0.27 | 0.44 |
| 11.Fish and seafood | 0.02 | 0.02 | 0.02 | 0.06 | 0.08 | 0.11 |
| 12.Eggs | 0 | 0.01 | 0.01 | 0.01 | 0.02 | 0.04 |
| 13.Milk and dairy-based products | 0.19 | 0.23 | 0.24 | 0.5 | 0.54 | 0.59 |
| 14.Miscellaneous / Food for special dietary uses | 0 | 0 | 0.01 | 0 | 0.01 | 0.05 |
| 15.Tap water | 0 | 0.17 | 0.52 | 0 | 0.62 | 1.38 |

b.w.: body weight; UB: Upper bound; min: minimum value; P95: 95$^{th}$ percentile; max: maximum value.

**Table 32:** Statistics on dietary exposure to cyanuric acid (µg/kg b.w. per day) calculated across the European countries for which consumption data at individual level are present in the Concise food consumption database using the P95 of the upper (UB) bound concentrations from the CIAA, CEPE and Empac joint dataset and the mean and P95 of consumption.

| Food category | Exposure statistics across countries based on MEAN of consumption µg/kg b.w. per day | | | Exposure statistics across countries based on P95 of consumption µg/kg b.w. per day | | |
|---|---|---|---|---|---|---|
| | min | median | max | min | median | max |
| 01.Cereals & cereal products | 0.41 | 0.66 | 0.82 | 0.78 | 1.27 | 1.67 |
| 02.Sugar & sugar products including chocolate | 0.03 | 0.06 | 0.11 | 0.09 | 0.16 | 0.25 |
| 03.Fats (vegetable and animal) | 0.02 | 0.05 | 0.09 | 0.07 | 0.12 | 0.2 |
| 04.Vegetables, nuts, pulses including carrots, tomato and leafy vegetables | 0.84 | 1 | 1.47 | 1.93 | 2.36 | 3.78 |
| 05.Starchy roots or potatoes | 0.13 | 0.26 | 0.74 | 0.35 | 0.63 | 1.79 |
| 06.Fruits | 0.1 | 0.17 | 0.41 | 0.42 | 0.54 | 1.23 |
| 07.Fruit and vegetable juices, soft drinks and bottled water | 1.13 | 1.53 | 2.68 | 3.71 | 5.1 | 9.45 |
| 08.Coffee, tea, cocoa (expressed as liquid) | 0.09 | 0.39 | 0.61 | 0.26 | 0.9 | 1.35 |
| 09.Alcoholic beverages | 0.09 | 0.1 | 0.1 | 0.48 | 0.55 | 0.62 |
| 10.Meat and meat products, offal | 0.39 | 0.52 | 0.55 | 0.93 | 0.94 | 1.52 |
| 11.Fish and seafood | 0.03 | 0.04 | 0.05 | 0.13 | 0.17 | 0.23 |
| 12.Eggs | 0 | 0.01 | 0.02 | 0.03 | 0.04 | 0.1 |
| 13.Milk and dairy-based products | 0.89 | 1.09 | 1.17 | 2.39 | 2.58 | 2.83 |
| 14.Miscellaneous / Food for special dietary uses | 0.01 | 0.03 | 0.08 | 0.03 | 0.06 | 0.28 |
| 15.Tap water | 0 | 0.2 | 0.6 | 0 | 0.72 | 1.6 |

b.w.: body weight; UB: Upper bound; min: minimum value; P95: 95th percentile; max: maximum value.

The data show that the exposure is mainly driven by consumption, like in the case of water, vegetables and cereals. Category "07.Fruit and vegetable juices, soft drinks and bottled water" appears to have a moderately higher contribution than other "high consumption" categories. These values are considered conservative, because the occurrence data are in most cases upper bound values for not detected samples, as discussed in chapter 4.1.

### 5.3. Exposure assessment for melamine and cyanuric acid in infants

Melamine and cyanuric acid occurrence in infant formulas are part of the category "14B.Food for special dietary uses" in Table 5 and Table 17. In particular, infant formulas were represented by 565 samples in the melamine sub-set and 203 samples in the cyanuric acid sub-set. All the reported values are extremely low, with maxima at 0.05 mg/kg for melamine and 0.03 mg/kg for cyanuric acid. The P95 is at 0.01 mg/kg and 0.03 mg/kg, respectively. These values most probably represent the limit of quantification of non detected samples. A worst case exposure scenario may be calculated using the P95 values and the consumption levels described in chapter 5.1.2.2., for a 6 kg infant.

The estimated exposures to melamine based on the P95 upper bound occurrence level are 1.3 µg/kg b.w. for average consumers and 1.8 µg/kg b.w. for high consumers. The estimated exposures to cyanuric acid based on the P95 upper bound occurrence level are 4 µg/kg b.w. for average consumers and 5.5 µg/kg b.w. for high consumers. These values represent a conservative estimate of the exposure of infants to melamine and cyanuric acid based on the analytical data on infant formulas provided by the industry, applying an approximate, empirical dilution factor of 8 (1 part of powdered formula+7 parts of water).

## 6. Hazard identification and characterisation

An update of the literature related to melamine toxicokinetics and toxicity in laboratory animals, farm animals, pets, fish and humans, was performed using literature searches (1966-March 2010) in selected databases (Pubmed, Medline, Web of Science).

### 6.1. Toxicokinetics

There are limited data on the toxicokinetics of melamine and its analogues. There are some data on bacterial metabolism and on the toxicokinetics in rats, pigs and cows. Additional information can also be found in the JMPR assessments of the pesticide cyromazine (JMPR 1991, 2008). For the analogues, data on toxicokinetics are only available for (iso)cyanurate, as this substance is a breakdown product of the biocide dichloroisocyanurate. However, the toxicokinetic data on this substance were only published in two abstracts and in a review paper. The available data have been presented in the sections below. In a few studies melamine has been identified as a component of urinary tract deposits (e.g. in sheep) without any further data on toxicokinetics. As such studies also show urinary elimination of the substance, these studies have also been included in this chapter.

#### 6.1.1. Melamine

##### 6.1.1.1. Bacterial metabolism

Melamine metabolism has been studied in bacterial cultures (*Klebsiella* and *Pseudomonas*). Melamine was added as the sole source of nitrogen. Various strains of both species were able to deaminate melamine to ammeline, ammelide and cyanuric acid. Following hydrolytic ring cleavage cyanuric acid was converted into $NH_4^+$ and carbon dioxide; probably via biuret and urea (Jutzi et al., 1982; Shelton et al.,1997). Deamination is a common pathway of rumen bacteria and protozoa and hence ruminal biotransformation of melamine can be expected. However, detailed studies are not available (Wallace, 1996).

##### 6.1.1.2. Laboratory animals

Lipschitz and Stokey (1945) reported that rats excreted at least 50 % of an oral dose of melamine in the urine. Based on elemental analysis of crystals obtained after urine sample clean-up, it was considered conceivable that melamine formed a co-precipitate with phosphate. It was not reported whether the melamine-phosphate precipitate was already present in the urine when excreted or whether the precipitate was formed upon standing. Similarly, it was demonstrated that in dogs at least 60-87 % of an oral dose of melamine was eliminated via the urine within 24 hours.

The metabolism, excretion and disposition of melamine was assessed after oral administration of a single dose of 0.025 mCi (~1.3 mg/kg b.w.) [$^{14}$C]-melamine to adult male Fischer 344 rats (Mast et al., 1983). Overall, *ca.* 90 % of the administered dose was excreted within the first 24 hours into urine, exhaled air and faeces, and at 96 hours post-dosing 93, 0.2 and 0.64 % of the dose were recovered from urine, exhaled air and faeces, respectively. Total recovery (including cage washings) was 99 %. No residual radioactivity (LOD: 1 µg/kg tissue) was observed in blood and plasma after 24 hours and at this time point concentrations of radioactivity in liver and kidneys were 1.8 and 1.3 µg equivalents/kg tissue, respectively. Radioactivity levels were much higher in the bladder and ureter (31 and 12 µg equivalents/kg tissue, respectively). The authors concluded that melamine distributes in body water. The relatively high levels in bladder were suggested to be probably due to either back diffusion from urine or to a contamination of the bladder tissue with urine. In a review of the paper by Mast et al. (1983), EFSA (2007) argued that also reabsorption of melamine in bladder or ureter could have happened. The elimination half-life derived from plasma data was 2.7 hours and was similar to the urinary-excretion half-life of 3.0 hours. Renal clearance of melamine was 2.5 ml/min. In addition to the blood and tissue levels, this rapid clearance from the body shows that melamine and its

analogues do not accumulate in mammalian tissues. Only one peak of radioactivity, with a retention time similar to melamine, was present in a reversed phase chromatogram of urine samples, together with a minor amount of radioactivity just before and after this main peak. In the urine at all time points studied the same fraction of total radioactivity (i.e. the radiochemical purity) was associated with melamine as in the dosing material. The same radiochemical purity was observed in radio-chromatographs from blood and plasma. In faeces *ca.* 70 % of the radioactivity was represented by the melamine peak. Considering the total radioactivity eliminated via this route (0.64 % of the dose), the non-melamine-$^{14}$C would comprise only 0.19 % of the dose. It was argued that no radioactivity was present in urine or plasma that was not also present in the dosing solution and therefore melamine is not metabolised by the male rat (Mast et al., 1983).

Intestinal absorption and urinary excretion of melamine was studied in male rats dosed with 1 mg of melamine, either via intravenous (i.v.) injection in the ligated stomach, or in a ligated part of the small intestine. Melamine was not absorbed from the stomach over a period of 30 minutes, but rapidly absorbed from the small intestine. A monoexponential increase in urinary melamine excretion was observed. A small amount of the absorbed melamine was suggested to be excreted in the bile and subsequently reabsorbed, based on secondary peaks in melamine blood levels (Sugita et al., 1991).

Melamine was administered in a single intravenous (2 mg/kg b.w.) or oral (5 mg/kg b.w.) dose to male Sprague–Dawley rats (presumably 3/group) and the toxicokinetic characteristics of melamine were investigated. After oral administration, melamine was rapidly absorbed from the gastrointestinal (GI) tract with a peak plasma level of 60.8 µg/ml at 1 h post dosing. The mean values of major first-order toxicokinetic parameters of oral availability, the mean steady-state distribution volume, plasma clearance, and plasma elimination half-life (T1/2) of melamine in Sprague–Dawley rats were 73 ± 13 %, 103 ± 13 mL/kg, 20 ± 4 mL/h/kg, and 4 - 5 h, respectively. The data suggested that melamine was predominantly restricted to blood or extracellular fluid and was not extensively distributed to most organ tissues (Yang et al., 2009). Log-linear reanalysis of the data from the intravenous exposure revealed that the kinetics might be better described with a second-order model. The half-life in the initial phase could be estimated at approximately 1.5 h. The plasma elimination half-life in the second phase could be estimated at approximately 6 h.

Indirect evidence that melamine is not metabolised in the rat can be inferred from a study in which [$^{14}$C]-ring-labelled hexamethylmelamine (an antineoplastic agent) was administered to rats (~ 25 mg/kg b.w.; intraperitoneal (i.p.)). Expired air, urine and faeces were collected for analysis of radioactivity. Urine samples were further examined using gas chromatography. Hexamethylmelamine was demethylated in 6 subsequent steps leading to melamine. Approximately 2 % of the dose of hexamethylmelamine (as $^{14}$C) was excreted as melamine in rat urine. No metabolites of melamine were reported, but 5 % of the dose of hexamethylmelamine (as $^{14}$C) was found in the urine as unidentified metabolites. No exhalation of radioactivity was observed (Worzalla et al., 1974). No release of $^{14}CO_2$ was seen, which might indicate absence of deamination and subsequent ring opening. No detailed study was made on the occurrence of deamination products of melamine in the urine (in particular ammelide, ammeline or cyanuric acid). These substances may have been present in the unidentified fraction (*ca.* 5 % of urinary radioactivity in the rat).

Melamine was orally administered by gavage to three fasted Rhesus monkeys (average body weight: 5.8 kg; one female, two males) at a single dose of 1.4 mg/kg b.w. as a solute in glycerol (Liu et al., 2009). The selected dose was equivalent to the SCF TDI of 0.5 mg/kg b.w., allometrically scaled from human to monkey based on body surface ratio. Plasma and urine were collected for the determination of melamine and cyanuric acid with a LC-MS/MS method, under conditions suitable to take account of possible insoluble melamine-cyanuric acid co-precipitates. The mean ± standard deviation (SD) area under the concentration-time curve from time zero to 48 h (AUC0-t) was 14145 ± 2002 µg/L×h. The maximum concentration of melamine in plasma (Cmax) was 1767 ± 252 µg/L. The time to maximum concentration (Tmax) was 2.67 ± 1.16 h and the half-life of melamine in plasma (t1/2) was 4.41 ± 0.43 h. At 36 h post dosing no melamine could be found in the plasma (LOQ: 10 ng/ml). No significant correlation was found between melamine and cyanuric acid levels in plasma or urine,

suggesting that cyanuric acid may not be derived from melamine. Biochemical parameters for liver and kidney function in plasma and urine were also studied. No changes in any of these parameters were reported, but detailed documentation on clinical chemical methods or findings was not provided. According to the study authors, melamine was rapidly excreted following oral administration, mainly through urinary clearance (Liu et al., 2009). This statement cannot be supported from this study because, when the documentation on urinary excretion is used to calculate a mass balance, a large fraction of the dose is not accounted for in the urinary excretion profile.

#### 6.1.1.3. Humans

No direct data on the toxicokinetics of melamine have been located. From the recent melamine intoxication episode, it is clear that melamine will be excreted in human urine.

Indirect evidence that melamine is not metabolised in humans can be inferred from a study in which [$^{14}$C]-ring-labelled hexamethylmelamine (and antineoplastic agent) was administered to humans (4 mg/kg b.w.; per os (p.o.)). Expired air, urine and faeces were collected for analysis of radioactivity. Urine samples were further examined using gas chromatography. Hexamethylmelamine was demethylated in 6 subsequent steps leading to melamine. Five percent of the dose of hexamethylmelamine (as $^{14}$C) was excreted as melamine in human urine. No metabolites of melamine were reported. No exhalation of radioactivity was observed (Worzalla et al., 1974). No release of $^{14}CO_2$ was seen, which might indicate absence of deamination and subsequent ring opening. No detailed study was made on the occurrence of deamination products of melamine in the urine (in particular ammelide, ammeline or cyanuric acid). These substances may have been present in an unidentified fraction (*ca.* 5 % of urinary radioactivity).

#### 6.1.1.4. Farm animals

##### 6.1.1.4.1. Ruminants

Melamine has been suggested as a non-protein nitrogen feed additive for ruminants in the past. This form of nitrogen supplementation was shown to be effective only in low protein feed. The efficacy further decreased as a result of feed intake reduction and loss of physical condition (e.g. MacKenzie, 1966). It has been studied if melamine would release ammonia in the rumen of cattle *in vitro* and in vivo (Newton and Utley, 1978). Addition of melamine to cattle feed resulted in an increase of rumen ammonium concentration after an adaptation period. A nitrogen balance calculation showed that only ~ 6 % of the melamine nitrogen was retained. The authors speculated that the ring-nitrogens would not be available for protein synthesis. In the *in vitro* study in rumen fluid it was shown that melamine-adapted rumen fluid was able to release melamine nitrogen to some (minor) extent. The release of nitrogen from melamine in ruminants was considered to be related to microbial activity. Although melamine has been found in tissues and in milk of several species (for overview see Tolleson et al., 2009), only one systematic study seems to have been published in which carry-over of melamine to milk has been examined. Cruywagen et al. (2009) dosed dairy cows with (nominally) 17.13 g of melamine per day via the feed. The substance was given in 15 kg of a pelleted feed supplement, which contained 1142 mg melamine/kg. Additionally oat hay fodder was provided. Animals were milked twice daily and afternoon milk samples were analysed for melamine (the first sample was taken 8 hrs after the first morning milk; sampling interval time was 24 h). Melamine appeared in the milk within 8 hr following the first feeding and reached a plateau value of approximately 15 mg/kg milk at 56 h. Upon cessation of exposure, milk melamine levels initially declined rapidly (by 39 % at 8 hrs and by 85 % at 32 hrs post exposure) but fell below the detection limit (5 µg/kg of milk) only after 6 days. Apart from normal daily variation, milk yield remained constant during the experiment at approximately 25 kg per day Average total melamine excretion via the milk can be calculated at $15 \times 25 = 375$ mg per day, during steady-state (Cruywagen et al., 2009). From these data a steady state transfer of 375 / 17.13 = 22 mg melamine in milk per gram melamine in the feed (2.2 %) can be

calculated. This calculation is worst-case as the rapid renal elimination of melamine will concomitantly reduce the transfer of melamine from plasma to milk.

6.1.1.4.2. Monogastric animals

**Poultry**

An experiment was conducted to determine melamine residue levels in the tissues of broiler chickens fed diets containing graded levels of melamine (Lu et al., 2009). Ten experimental diets were developed to contain 0, 2, 5, 10, 20, 50, 100, 200, 500, and 1,000 mg of melamine/ kg of diet. Each diet was offered in 4 replicate cages (12 male 1-day old broiler chickens per cage) from day 1 to 42, followed by a 7-day feeding of a withdrawal diet that contained no melamine. Throughout the 42-day feeding period, no effect on weight gain, feed intake, feed conversion ratio, and survival of broiler chickens was observed.

On days 28, 42, and 49, one bird per replicate cage was killed and tissue samples from the breast meat, liver, and kidney were collected for the determination of melamine residues. Residue levels of melamine in broiler tissues at days 28 and 42 were below the detection limit (LOD 2 mg/kg tissue) when the diets contained ≤50 mg/kg of melamine. At day 28, tissue melamine levels increased ($P < 0.05$) with the increasing levels of melamine in birds fed diets containing 100 mg/kg or more. At day 42, a similar trend of a dose-dependent increase in tissue levels was observed in the kidney, but melamine was detected in breast meat and liver only in birds fed diets containing 500 and 1,000 mg of melamine/kg of diet. Also, the melamine levels in breast meat, liver and kidneys were much lower after 42 days of feeding as compared to the levels observed after 28 days of feeding. The authors postulated that this could be explained by an increased melamine clearance with increasing age, but it may also reflect a decreased exposure to melamine on a mg/kg b.w. per day basis following the feeding behaviour of the growing animals. In addition some of the differences between day 28 and day 42 might be due to variability in time of sampling. If melamine has a similar short half-life in chicken as it has in rats, then the time gap between sampling and feeding is crucial for the determination of tissue levels. However, feed intake and body weight data were not provided so the actual exposure during the study cannot be calculated. Melamine distribution varied ($P < 0.05$) in different tissues, with the highest concentration in the kidney. A withdrawal period of 7 days was found to clear the tissues of melamine. The reported levels in the broiler tissues are presented in Table 33.

**Table 33:** Melamine concentrations in tissues of broilers fed melamine.

| Added melamine in feed (mg/kg) | Melamine concentration in tissues (mg/kg tissue) | | | | | |
|---|---|---|---|---|---|---|
| | Day 28 | | | Day 42 | | |
| | breast meat | liver | kidney | breast meat | liver | kidney |
| 0 - 50 | ND | ND | ND | ND | ND | ND |
| 100 | ND | 2.73 | 4.33 | ND | ND | 1.74 |
| 200 | 1.81 | 2.76 | 6.08 | ND | ND | 3.17 |
| 5000 | 3.82 | 5.54 | 13.35 | 1.74 | 1.34 | 4.10 |
| 1000 | 8.04 | 9.70 | 29.52 | 3.73 | 2.70 | 9.17 |

Average values from 4 animals per tissue; ND: not detected (LOD 2 mg/kg tissue; reported levels < 2 mg/kg result from averaging data from separate animals. A method to include non-detects in this process was not provided.

On average, from the data in the table, transfer factors of 0.6, 1.3 and 2.6 % (mg/kg tissue per mg/kg feed) for breast meat, liver and kidney can be calculated for the 28-day exposure. For the 42-day exposure these factors would be 0.2, 0.1 and 1.1 % (mg/kg tissue per mg/kg feed) for breast meat, liver and kidney, respectively (Lu et al., 2009). It should be noted that melamine is not used (or licensed) as a feed additive for poultry in Europe.

At the estimated exposures presented in Table 19, with scenarios of melamine concentrations of 0.5, 2.5 and 10 mg/kg feed, corresponding to the lowest exposure level of 0-50 mg/melamine added to feed (Table 33), no measurable residues are expected in any organ of a broiler.

In a recent study (Chen et al., 2010), the disposition profile of melamine was established in laying hens, with special focus on the transfer of melamine to eggs. In this study, laying hens were given an experimental feed spiked with 0, 5, 25, 50 or 100 mg of melamine per kg of feed. Eggs were collected over a period of 15 days and analyzed for the presence of melamine by a validated GC/MS method. Melamine concentrations in eggs were 0.16, 0.47, 0.84 and 1.48 mg/kg egg, respectively, in the 0, 5, 25, 50 and 100 mg/kg feed treated groups. There was no evidence for a further accumulation of melamine in the egg yolk following long-term exposure and the calculated transfer rates vary between 1.5 and 3.2 %.

**Pigs**

Toxicokinetic data for melamine in pigs following intravenous bolus administration were obtained by Baynes et al. (2008). Melamine was administered at 6.13 mg/kg b.w. to weanling pigs (n=5) and plasma samples were collected over 24 h post administration. Plasma melamine concentrations could be described with a first-order one-compartment model with a half-life of 4.04 h, a clearance of 0.11 L/h/kg and a volume of distribution of 0.61 L/kg. The data from Mast et al. (1983), Sugita et al. (1991) and Baynes et al. (2008) were used to build a multi-compartment toxicokinetic model which could predict levels of melamine in plasma, liver and kidney tissue in pigs exposed to melamine via the food (Buur et al., 2008).

The model consisted of four tissue compartments that include kidney, liver, plasma, and carcass, which represents the combination of the remaining tissues (Figure 6). Melamine input was modeled as intravenous directly into the plasma compartment. In addition concentration time curves for repeated oral dosing were generated with a model expanded to describe absorption from the GI-tract. Figure 6 depicts a schematic diagram of the chronic oral dosing module which consisted of two sub-compartments representing the stomach and small intestine (site of absorption). All melamine was assumed to be instantaneously available in the stomach. Transport into the small intestine was controlled by the gastric emptying time (Kst) and assumed to be first-order. Absorption into the liver compartment was governed by first order kinetics with constant Ka. Implicitly, 100 % bioavailability was assumed, which is in line with the very high renal excretion as reported in the rat (Mast et al., 1983). Physiological constants for organ volume, tissue blood volume, and blood flow for male Fischer 344 rats were obtained from various literature sources. Based on the experimental data from the rat, hepatic clearance was considered insignificant and total body clearance was assumed to be by renal elimination only. Renal clearance was modeled as first-order excretion with constant Clrenal. Model parameterization and optimization was against data from *in vivo* studies in rats (Sugita et al., 1991) and the model was subsequently validated against the *in vivo* study by Mast et al. (1983).

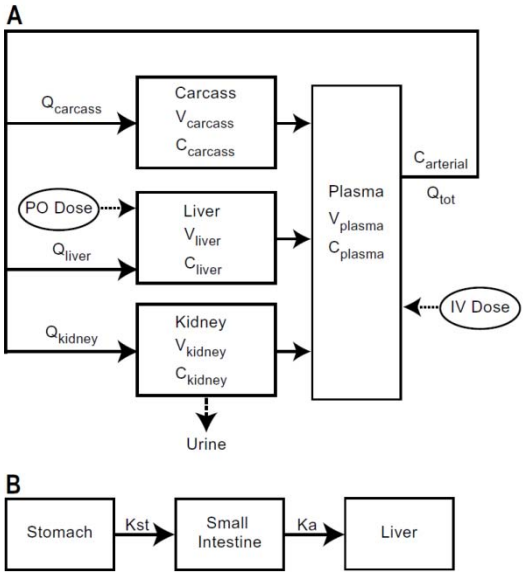



**Figure 6:** Schematic illustration of the physiologically based pharmacokinetic model of melamine (A). This model was used in both rat and porcine species. Arrows represent mass transfer of melamine via blood flow. (B) Schematic representation of the chronic oral dosing regimen. Kst and Ka are rate of gastric emptying and rate of absorption, respectively (Figure taken from Buur et al., 2008).

The model was subsequently applied to pigs using literature data for pig physiology and various blood-tissue partitioning coefficients as estimated from the studies in rats and validated against the concentration-time data from the study in pigs by Baynes et al. (2008), in which melamine was administered intravenously. The model from Buur et al. (2008) was also re-implemented by RIVM-RIKILT (2008) (see Appendix I). (Figure 7).

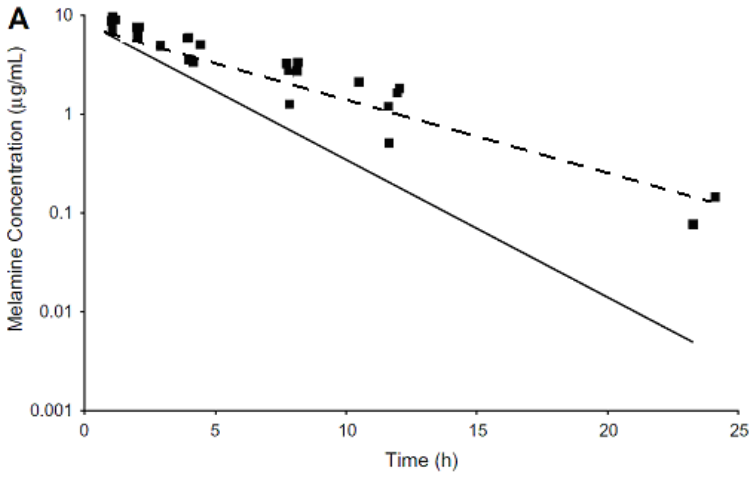



**Figure 7:** Validation data for porcine melamine physiologically-based pharmacokinetic modelling (PBPK) model against porcine plasma data. Solid line: Model prediction by Buur et al. (2008); Dashed line: model prediction by RIVM-RIKILT (2008); Squares: observed data (Baynes et al., 2008).

RIVM-RIKILT (2008) have applied the modified porcine PBPK model to an example case to experimental data for which animals of 40 kg body weight are exposed to feed contaminated with 500 mg melamine per kg feed, fed in portions of one kg twice a day (i.e. in total 1000 mg/animal per day) for seven days. This regimen implies intake of 12.5 mg melamine per kg body weight twice a day. Tissue concentrations after intravenous injection of melamine (12.5 mg/kg b.w.; twice daily for seven days) was also modelled, to assess sensitivity to the model parameters. For the prediction of melamine concentrations in muscle tissue, it was assumed that these were adequately reflected by the concentrations in the carcass compartment (see Figure 8). The model simulation of the concentrations in muscle after oral and intravenous administration are shown in Figure 8. Concentrations in liver, kidney and plasma follow the same pattern. Note that in this situation a "plateau" is reached because the minimal and maximal concentrations are constant after ca 25 hours. Due to the short half-life considerable daily variation in plasma and tissue melamine levels can be anticipated.

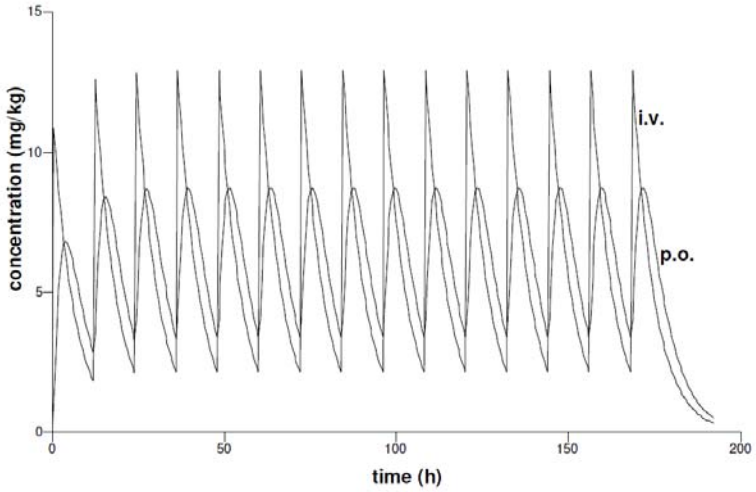



**Figure 8:** PBPK simulation of the time-course of melamine in muscle tissue of pigs (40 kg) exposed twice daily to 500 mg/kg melamine in feed (i.e. 12.5 mg melamine/kg body weight) or exposed twice daily to the same dose intravenously, during seven days (figure taken from RIVM-RIKILT, 2008).

**Table 34:** Concentrations and transfer factors of melamine after reaching plateau. Data obtained for pigs after an oral administration of 12.5 mg/kg b.w. twice daily in feed for 7 days (data taken from RIVM-RIKILT, 2008).

| | **muscle** | | **liver** | | **kidney** | |
|---|---|---|---|---|---|---|
| | C | TF | C | TF | C | TF |
| Minimum | 3.5 | 0.7 | 7.2 | 1.6 | 10.8 | 2.2 |
| maximum. | 8.7 | 1.7 | 18.9 | 3.8 | 26.8 | 5.4 |

C: concentration in mg/kg; TF: transfer factors expressed in percentage (%).

Minimum and maximum melamine concentrations in liver, kidney and muscle tissues after reaching "plateau" following repeated oral administration are presented in Table 34. Muscle, liver and kidney concentrations (mg/kg) ranging from 3.5 to 8.7 (muscle), 7.2 to 18.9 (liver) and 10.8 to 26.8 (kidney) could be calculated. Corresponding transfer factors (calculated for the dose interval and defined as tissue concentration/feed concentration*100 %) were 0.7 to 1.7 % (muscle), 1.6 to 3.8 % (liver), 2.2 to 5.4 % (kidney) and 1.0 to 2.5 % (plasma). Note that separate upper and lower transfer factors were calculated for the high and low plasma or tissue concentrations, respectively, and that estimation of transfer factors is somewhat artificial due to the short half-life of melamine and the subsequent high variability of the plasma and tissue values. For the interpretation of these findings, it should be noted that melamine is not indicated or licensed for use in pigs.

6.1.1.5. Pets

There are no specific data on the toxicokinetics of melamine in pet animals such as cats and dogs, despite the detailed description of the renal pathology from the pet feed contamination incident.

### 6.1.2. Melamine precursors: Cyromazine

The insecticide cyromazine (= N-cyclopropyl-melamine) is metabolised by rats, monkeys, goats, hens and sheep and various plants to melamine but only to a minor extent (JMPR, 1991, 2008).

Toxicokinetic studies in rats given [U-$^{14}$C]-triazine-ring-labelled cyromazine as single or repeated oral doses showed that it is rapidly and nearly completely absorbed from the gut, distributed to all organs and tissues and excreted rapidly into the urine (97 % of the dose after 24 hours). Cyromazine is incompletely metabolized via methylation, hydroxylation or N-dealkylation. In both faeces and urine, the parent compound represented 71 – 72 % of the radiolabel (*ca.* 63 % of the dose) and melamine represented only 7 % (6.5% of the dose), the remaining 20 % were hydroxycyromazine (*ca.*7 % of the dose) methylcyromazine (*ca.* 2 % of the dose) and several unidentified metabolites (together *ca.* 7 % of the dose). In monkeys (*Macaca fasicicula*[35]), [U-$^{14}$C]-triazine-ring-labelled cyromazine excretion was also rapid and extensive, representing 95 % of urinary radioactivity, the remaining 5 % radioactivity was attributed to melamine. In goats a similar fraction of the dose was converted to melamine. For sheep the data do not allow for an estimation of the fraction of the dose that is converted to melamine. Although further metabolites of melamine were not mentioned, it cannot be completely excluded that such biotransformation might occur. JMPR (1991, 2008) did not report on possible exhalation of radioactivity. The nature of 6 % of the radioactive dose excreted in urine or faeces was not identified.

Also in hens, after administration of [U-$^{14}$C]-triazine ring-labelled cyromazine, melamine residues could be demonstrated but the study summaries provide too limited detail to allow for a reliable estimation of total conversion. However, as about 80 % of the radioactivity was recovered in the excreta as parent substance total metabolism was limited. Radioactivity in various tissue samples was predominantly associated with cyromazine. Less than 10 % of tissue radioactivity was identified as melamine. It was stated that egg white contained a metabolite which was tentatively identified as ammeline. In hens no conversion to $^{14}CO_2$ was observed (JMPR, 1991).

Cyromazine is also used as a veterinary drug for fly-control. From the evaluation published by EMEP (2001) the following information has been extracted. The fate of cyromazine was investigated in a radiometric study in sheep. The animals were treated dermally with a pour-on formulation at a dose of 82 mg ring –labelled $^{14}$C-cyromazine/kg b.w. The animals were slaughtered at 2, 6 or 10 days after treatment, A run-off from the dose site of 28 % of the total dose on average was observed. Peak plasma levels of radioactivity were observed at 24 hours after treatment, and declined biphasically thereafter.

The mean levels of radioactivity in the tissues were highest (1.15 mg cyromazine equivalents/ kg) in fat from below the treatment area at 6 days after treatment, declining to 1000 µg/kg at 10 days. Highest mean radioactivity of 0.84 mg/kg in muscle samples was observed at 6 days after treatment, declining to 0.24 mg/kg at 10 days. The mean levels of radioactivity in liver and kidney were highest at 2 days post dose, i.e. 0.230 mg cyromazine equivalents/kg in liver, declining to 0.22 and 0.15 mg/kg at 6 and 10 days, respectively, and 0.17 mg/kg in kidney, declining to 0.01 and 0.02 mg/kg at 6 and 10 days, respectively.

The parent compound cyromazine was the main residue present in muscle (86 %), fat (95 %) kidney (77 %), urine (96 %) and faeces (95 %). The residue composition in these matrices was constant in time. Melamine was found in some samples of muscle, fat and urine (up to 1 % of radioactive residues

[35] This Latin name does not exist. Probably *Macaca fascicularis* was meant: Cynomolgus monkey.

in these samples). The residue pattern in liver differed from that in the other tissues. There was no statement on the occurrence of melamine in liver, but cyromazine + N-methyl-cyromazine comprised 86 – 88 % of the total residue found. The overall picture of the residue profile in the edible tissues of sheep showed that the parent compound (cyromazine) is the most appropriate marker residue.

Cyromazine residues were investigated in sheep, one and seven days after dipping in a 1000 mg/L cyromazine solution. The results showed high variation in residual amounts of cyromazine muscle 170 to 1800 µg/kg at day 1, declining to 0.03 to 0.230 mg/kg at day 7, kidney 0.14 to 6 mg/kg declining to 0.08 to 0.9 mg/kg, liver 0.12 to 2.2 mg/kg, declining to 0.05 to 0.35 mg/kg and fat 0.27 to 0.72 mg/kg, declining to 150 to 310 µg/kg. Muscle was also analysed for melamine but this metabolite was not detectable (less than 0.05 mg/kg) (EMEP, 2001; original research reports not available to EFSA).

### 6.1.3. Cyanuric acid

#### 6.1.3.1. Laboratory animals

Upon oral administration to rats and dogs, $^{14}$C-cyanuric acid is rapidly absorbed and eliminated unchanged via the urine with elimination half-lives of 0.5 to 1 hr after 5 mg/kg b.w. (i.v. and p.o.) and ca. 2.5 hrs after 500 mg/kg b.w. (p.o.) for the rat. In the dog at both dose levels, an elimination half-live of 1.5-2 hrs was found. In both species the absorption at the high dose level appeared to be incomplete. The volume of distribution for cyanuric acid in the dog was 0.7 L/kg. In the rat, but not in the dog, a retarded elimination of cyanuric acid at the high dose level was observed. Traces of radioactivity were found in rat adrenal glands, fat, urinary bladder and intestines at 500 mg/kg b.w. at 7 days post dosing, but no appreciable bio-accumulation was anticipated in either species. In the rat no exhalation of $^{14}CO_2$ was seen and no metabolism of cyanuric acid was observed in rats and dogs. In the rat, repeated daily dosing with 5 mg/kg b.w. per day during 15 days did not result in major changes in the distribution of cyanuric acid (Barbee et al., 1983, 1984).

#### 6.1.3.2. Humans

Absorption and excretion of cyanuric acid has been studied in long-distance swimmers (9-17 years of age) exposed through 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 hours. Elimination could be described by a one-compartment open model, and a half-life of excretion of about 3 hours was calculated (Allen et al., 1982).

#### 6.1.3.3. Farm animals and pets

Apart from the observations in dogs as mentioned in section 6.1.3.1, data on the toxicokinetics of cyanuric acid in farm animals and pets have not been found in the literature, despite the various reports regarding toxic effects of contaminated pet food.

### 6.1.4. Ammeline and ammelide

No information is available on the toxicokinetics of ammeline and ammelide other than the observation that ammeline was tentatively identified as a metabolite of cyromazine in chicken eggs (JMPR, 1991). In a study in which melamine was added to the feed of broiler chicken at nominal concentrations up to 1000 mg/kg feed, no data were presented on the occurrence of ammeline or ammelide in tissues (Lu et al., 2009).

### 6.1.5. Toxicokinetic interaction between melamine, cyanuric acid and uric acid

6.1.5.1. Toxicokinetic interaction between melamine and cyanuric acid

Following extensive episodes of feed related poisoning in cats, dogs and other animals in the USA and other countries in 2004 and 2007, ample evidence has been generated that co-exposure to melamine and cyanuric acid results in the deposition of solids in the urinary tract (e.g. Brown et al., 2007; Reyers, 2007, 2008; Thompson et al., 2008; Dobson et al., 2008; Reimschuessel et al., 2009). These solids were identified as melamine-cyanuric acid co-crystals (Dobson et al., 2008). It was demonstrated that in vitro such co-crystals could be formed from melamine and cyanuric acid, but not from ammelide or ammeline with cyanuric acid. Cyanuric acid could only form co-crystals with ammeline or ammelide when solutions of these substances were vacuum-dried (Reimschuessel et al., 2008). In a sequence of studies in which melamine, including cyanuric acid, ammelide and ammeline were administered to rats, pets, pigs and 4 species of fish, acute exposure to high dose levels (400 mg/kg b.w.) of any of these substances did not result in the formation urinary tract deposits. The combination of melamine (400 mg/kg b.w. per day) and cyanuric acid (400 mg/kg b.w.per day) resulted in the formation of deposits in the renal tissues in fish and pigs (Reimschuessel et al., 2008).

The combination of melamine (400 mg/kg b.w. per day) and only 40 mg/kg cyanuric acid per day, in rats, resulted in the formation of deposits in the renal tissues (Dobson et al., 2008). It was discussed that in animals cyanuric acid, in addition to co-precipitating with melamine, might also inhibit urate oxidase, the enzyme that metabolises uric acid to allantoin. Melamine can also form urinary tract co-precipitates with uric acid and mixed solids have been found in some canine patients (Ogasawara et al., 1995; Dobson et al., 2008; Reimschuessel et al., 2008).

6.1.5.2. Toxicokinetic interaction between melamine and uric acid

Ogasawara et al. (1995) studied the composition of deposits in the bladders of rats given a diet containing 1 % melamine (equivalent to 500 mg/kg b.w. per day) for 40 weeks. These precipitates contained melamine and uric acid (61 – 81 %) in a 1:1 molar ratio. The full composition of the precipitates was not identified. The crystals were only reported to occur after 40 weeks of exposure but no clear data were presented on the time-course of their development. In other studies, different compositions of kidney or bladder stones in animals have been reported (e.g. melamine-phosphate co-precipitates; in Lipschitz and Stokey (1945) or melamine-protein co-precipitates in Heck and Tyl, (1985)). However, in many short-term studies with high dose levels, precipitation of melamine with other ions (such as phosphate) may have overwhelmed or obscured the co-precipitation of melamine with uric acid.

Following the episodes of melamine nephrotoxicity in pets in the USA and South Africa, Reyers (2007, 2008) anticipated that humans might be more susceptible to melamine toxicity than rodents or dogs. This suggestion was based on the composition of the urinary tract deposits reported by Ogasawara et al. (1995) and the lack of urate oxidase in humans, which results in higher uric acid excretion and potential for increased formation of melamine-uric acid crystals compared with other mammals. The reported ranges of uric acid concentrations in plasma and urine of a number of species are shown in Table 35. In addition, Table 35 shows that the urinary pH in humans can be lower than in rodents. The urinary pH in an adult human nephron can change from pH 7.4 in the Bouwman space, to pH 6.8 at the end of the proximal tubules, to pH 6.0 at the end of the inner medullary collecting duct, reaching a pH of 5.4 in the final urine (Giebisch and Windhager, 2003). In the healthy individual, urinary pH is controlled by various transport mechanisms for acids and bases. Diurnal variations in urine pH in normal subjects have shown that urine pH is approximately 6.0 for the majority of a 24-hour period. However, in infants and under conditions of metabolic acidosis, urinary pH can be as low as 4.4 (Battle and Shah, 2007). As discussed in section 1.3, formation of melamine-uric acid is more likely to occur in urine at pH values < 5.5 (Grases et al., 2009).

**Table 35:** Uric acid levels in plasma and urine and urinary pH from various species and humans[(1)].

| Species | Strain | Uric acid concentration mg/L | | | | Urinary pH |
|---|---|---|---|---|---|---|
| | | Plasma | | | Urine | |
| | | mean ± SD | | range | mean ± SD | range |
| | | Male | Female | | | |
| Mouse | "albino" | 41 ± 11 | 39 ± 10 | 12 – 50 | 41 ± 11 | |
| Rat | "albino" | 20 ± 3 | 18 ± 2 | 12 – 75 | 20 ± 3 | 7.3 - 8.5 |
| | 344/cr | | | 12 – 36 | | |
| | Osborne-Mendel | | | 18 – 30 | | |
| | BLU:(LE) | | | 9 – 34 | | |
| | Axenic (germ-free) | | | 12 – 24 | | |
| Syrian golden hamster | | 46 ± 5 | 44 ± 5 | 18 – 53 | 45 ± 5 | |
| Guinea pig | | 35 ± 4 | 34 ± 4 | 13 – 56 | 34 ± 4 | |
| Rabbit | | 26 ± 9 | 26 ± 9 | 10 – 43 | 26 ± 9 | 7.6 - 8.8 |
| Gerbil | | | | 11 – 31 | | |
| Opposum | | | | 9 – 22 | | |
| Armadillo | | | | 1 – 8 | | |
| Chicken | | 53 ± 12 | 53 ± 14 | 25 – 81 | 53 ± 12 | |
| Cat | | 15 ± 2 | 13 ± 2 | 0 – 19 | 14 ± 2 | 6.0 - 7.0 |
| Dog | | 6 ± 11 | 4 ± 1 | 2 – 9 | 5 ± 2 | 6.0 - 7.0 |
| | Alsatian | | | 2 – 6 | | |
| | Beagle | | | 2 – 6 | | |
| | Labrador retriever | | | 2 – 8 | | |
| | Mongrel | | | 2 – 7 | | |
| Monkey | | | | | 10 ± 1 | 5.5 - 7.4 |
| Rhesus monkey | | 9 ± 1 | 13 ± 1 | 11 – 15 | | |
| Baboon | | | | 0.4 - 0.2 | | |
| Pig | | 12 ± 5 | 12 ± 5 | 1 – 19 | 12 ± 5 | 6.3 - 7.5 |
| Goat | | 7 ± 3 | 6 ± 3 | 2 – 11 | | 7.5 - 8.8 |
| Sheep | | 12 ± 7 | 12 ± 7 | 0 – 19 | 13 ± 7 | 7.5 - 8.8 |
| Cattle | | 13 ± 7 | 11 ± 7 | 0 – 27 | | 7.6 - 8.4 |
| Horse | | 10 ± 1 | 9 ± 1 | 8.5 – 10 | | 7.8 - 8.3 |
| Humans | | 54 ± 16 | 41 ± 15 | 28 – 88 | 54 ± 13 | 4.8 - 7.8 |

(1) data compiled by from Mitruka et al. (1977); for comparison: the molecular weight of uric acid is 168.11 D, hence: 10 mg/L is equivalent to 59.5 µM. SD: standard deviation.

During the melamine incident in China in 2008, bladder and kidney precipitates observed in children were further examined. The precipitates were analysed by liquid chromatography – mass spectrometry (LC-MS), gas chromatography - mass spectrometry (GC-MS) and infrared (IR)- Fourier spectroscopic analysis, and found to be composed of melamine and uric acid in a ratio of 1:1.2 to 1:2.1. No cyanuric acid was found (Sun et al., 2009a, b). These data were also available to WHO in 2009 as an unpublished report. In the China melamine incident, cyanuric acid was hardly detected in infant formulae and in the raw materials from which the tainted baby food was produced (Wu et al., 2009a, b). An extensive discussion on the consequences of the renal excretion of uric acid in humans and its contribution to melamine nephrotoxicity was included in the Technical report of the WHO (2009a). Based on an overview by Fathallah-Shaykh and Neiberger (2008) (see Table 36), the report stressed that renal excretion of uric acid in infants is much higher than in children or adults. This, together with the immaturity of the kidneys, might explain a specific sensitivity to melamine toxicity of this age group.

**Table 36:** Urinary excretion in neonates, children and adults [1]. The table presents the comparison of the serum uric acid concentrations and the fractional excretion in urine.

| | Neonates[2] | | | Children | | | | | | Adults | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 29-33 wk | 34-37 wk | 38-40 wk | 3-4 y | | 5-9 y | | 10-14 y | | 40-44 y | |
| | | | | M | F | M | F | M | F | M | F |
| Serum uric acid (mg/L) | 77[4]±27 | 60±22 | 52±16 | 35±10 | 34±8 | 36±1 | 37±9 | 43±12 | 41±12 | 51±13 | 43±11 |
| Uric acid excretion (mg/L GFR[3]) | 48±22 | 28±9 | 17±8 | 3±1 | | | | | | 4.3±1 | |
| Uric acid excretion (mg/kg per day) | N/A | N/A | 19.6 | 13.5±3.8 (3 y) | | 11.5±3.8 (7 y) | | 9±3.8 (12 y) | | 10 | |

[1] Data from neonates taken from Stapleton (1983); other data taken from Fathallah-Shaykh and Neiberger (2008), who quoted without giving a source reference; [2] Gestational age of neonates; [3] mg/L glomerular filtration rate (i.e. concentration in the primary urine; see Gillespie and Stapleton, 2004). [4] For comparison: the molecular weight of uric acid is 168.11 D. Hence: 10 mg/L is equivalent to 59.5 µM. wk: week; y: years; M: male; F: female; GFR: glomerular filtration rate..

Even in the absence of melamine, the higher excretion of uric acid by infants makes them more prone to formation of urinary stones (Stapleton, 1978, 1983; Fathallah-Shaykh and Neiberger, 2008; Gillespie and Stapleton, 2004). Amongst the factors that may affect the formation of uric acid stones is urinary pH. At higher pH, solubility of uric acid (having a pKa of 5.8) is increased, and therefore alkalinisation is used as a non-surgical therapy to remove uric acid stones (Gillespie and Stapleton, 2004; Fathallah-Shaykh and Neiberger, 2008).

With regard to the complex formation between melamine and uric acid, the dissociation constant $k_d$ for the melamine-uric acid complex M-UA was measured by Tollesson over the pH range 4 to 7 (Tollesson et al., 2009).

$$\text{M-UA} \;<\!\!-\!\!>\; \text{M} + \text{UA}$$

The dissociation constant $k_d$ is defined as [M]x[UA]/[M-UA] and so the molar ratio of free melamine to complexed melamine [M]/[M-UA] can be calculated from $k_d$/[UA]. The values of $k_d$ at pH 7.9 and pH 5.5 can be extrapolated and interpolated respectively from the Tollesson data giving estimates of $1.4 \times 10^{-3}$ M and $3.2 \times 10^{-4}$ M respectively.

Taking for rat urine a pH of 7.9 (being the mid-point of the pH range in Table 22) and a concentration of uric acid of 20 mg/L (Table 22) or $1.19 \times 10^{-4}$ M for a molecular weight of 168, then the ratio of free melamine to complexed melamine can be estimated to be 11.8, meaning that 7.8 % of total melamine is complexed with uric acid in the rat urine.

Taking for human urine a pH of 5.5, this being the pH at which the melamine-uric acid complex is least soluble (Tollesson et al., 2009; Dominguez Estevez et al., 2010) and a concentration of uric acid of 54 mg/L (Table 34) or $3.21 \times 10^{-4}$ M, the ratio of free melamine to complexed melamine can be estimated to be 1.00, meaning that 50 % of total melamine is complexed with uric acid in the human urine.

The urinary concentration of uric acid for neonates at gestational age 38-40 weeks is 4-times higher than for adults (17/4.3, Table 35) and so using these equations the ratio of free melamine to complexed melamine can be estimated to be 0.25, meaning that 80 % of total melamine is complexed with uric acid in the urine of infants.

These calculations assume that the concentration of free uric acid in urine (UA) is undiminished from the total excretion values in Tables 34 and 35 by any complexation with melamine. This is a conservative assumption. Any such decrease in UA would cause a lower proportion of melamine to be complexed and the magnitude of any effect will depend on the value of $k_d$ along with the dose of melamine in proportion to uric acid.

With 7.8 % of melamine estimated to be complexed with uric acid in rat urine and 80 % estimated to be complexed in the urine of infants, there is therefore an approximately 10-fold higher propensity for the melamine-uric acid complex to form in infants than in rats, as a result of the lower pH and the higher excretion of uric acid.

**Summary of toxicokinetics**

Based on a limited number of studies, it can be concluded that in animals melamine is rapidly absorbed from the GI-tract and rapidly eliminated from the body with a plasma-half-life of a few to several hours. The major route of elimination is via the urine, and the limited information available indicates that the substance is hardly metabolised, if at all. Analytical techniques for identification of metabolites in urine have not been published in sufficient detail to exclude that a small fraction of an oral dose could be metabolised. In some studies unidentified fractions in urine have been reported. In other studies analytical techniques for the identification of melamine or metabolites may not have been sufficiently specific or specificity could not be evaluated. However, complete breakdown of melamine to $CO_2$ does not seem to occur.

No significant accumulation of melamine in tissues is anticipated. Based on toxicokinetic modelling, and on the results from the available studies, levels in tissues may be anticipated to closely follow plasma levels. Transfer factors for melamine to pig meat, liver and kidney are approximately 2 % (calculated as ratio of concentration in tissues and concentration in feed), but are very dependent on the moment of sampling due to the rapid elimination of melamine. Melamine has been demonstrated to be transferred to cow's milk. As a worst-case estimate it can be calculated that, after repeated daily exposure, about 2 % of the daily dose will be transferred to milk. In a feeding experiment with chicken the presence of melamine in chicken meat, in liver and in kidneys has been observed. Although the data from this study show some variability, the highest estimated transfer factor for chicken meat would amount to 0.6 % (mg/kg meat per mg/kg feed). Chicken kidneys may show a higher transfer factor (up to 2.6 %), but as these organs are embedded in deep bony crypts of the pelvic and synsacral area of the skeleton[36], they need not be considered in the human exposure estimation.

Melamine is a known metabolite of the insecticide and veterinary drug cyromazine. Data available for the veterinary use of cyromazine in sheep suggest that residue levels of melamine in meat would not be higher than 1 % of the total residue when this is equal to the MRL for cyromazine.

The limited information available for cyanuric acid also indicates rapid absorption from the GI tract and rapid elimination via the urine with little or no biotransformation. Also for this substance plasma half-lives of maximally a few hours have been reported.

For the two related substances, ammeline and ammelide, no data on kinetics have been found other than the observation that ammeline was tentatively identified as metabolite of cyromazine in chicken eggs.

Exposure to melamine is known to result in formation of deposits in the urinary tract, especially if co-exposure to cyanuric acid occurs. The co-precipitates of melamine and cyanuric acid are acid labile and may dissolve upon acidification of the urine. Under conditions of normal urinary pH the co-precipitates are stable.

[36] See: U.S. Department of Agriculture Food Safety and Inspection Service; http://www.fsis.usda.gov/PDF/PSIT_Anatomy.pdf

In rats and in humans melamine may also co-precipitate with uric acid and it has been discussed that humans may be more susceptible for this co-precipitation than many other animal species (except chicken) because humans excrete more uric acid in the urine than most mammals owing to a lack of the enzyme urate oxidase. In neonates, the excretion of uric acid in the urine is even higher than in adults and for that reason it can be argued that babies will be even more sensitive to melamine nephrotoxicity than adults. In a recent episode of large scale melamine contamination of infant formula, urinary tract deposits were shown to consist of melamine and uric acid. A factor that may further influence an individual's sensitivity to melamine is a higher urinary acidity, which will increase the likelihood of melamine-uric acid co-crystal formation in the urinary tract.

It is not known what concentrations of melamine and cyanuric acid should be present in urine to result in formation of the complex and subsequent formation of deposits in the urinary tract. It is also not known what urinary concentrations of melamine and uric acid should be present, to result in the formation of melamine-urate co-crystals. It has been suggested that a urinary concentration of 7.1 μg melamine /mmol creatinine predicts predisposition for melamine-related urinary tract deposits with a specificity and a sensitivity of 87 % and 60 %, respectively, but this value has been suggested without cut-off values for the other relevant urinary parameters (in particular pH and uric acid concentration).

## 6.2. Toxicity data in laboratory animals

### 6.2.1. Melamine

#### 6.2.1.1. Acute toxicity

Melamine has low acute toxicity. The lowest oral 50 % lethal dose ($LD_{50}$) was found in male rats (3828 mg/kg b.w.). The $LD_{50}$ was quite similar in female rats and male mice, but higher in female mice (NTP, 1983; Melnick et al., 1984).

#### 6.2.1.2. Short-term studies

F344/N rats of age 11 weeks were given diets with 0, 5000, 10 000, 15 000, 20 000, or 30 000 mg of melamine/kg for two weeks. All animals survived to the end of the study. Dose-related weight reduction was observed in rats receiving feed with 15 000 mg/kg or more. A hard crystalline solid was found in the urinary bladder in 4-5/5 male rats in groups fed 10 000 mg/kg or more, and in 4/5 female rats fed 20 000 mg/kg or more. Pale and pitted kidneys were observed in 2/5 males receiving the highest dose. The same concentrations in diets were fed to B6C3F1mice. All 5 males and 2/5 females receiving the highest dose had a hard crystalline solid in the urinary bladder (NTP, 1983).

#### 6.2.1.3. Sub-chronic studies

**Rats**

Three separate 13 week studies have been conducted in F344 rats that were 5-6 weeks old at start of experiment. In the first study the rats (12 of either sex) received feed containing 0, 6000, 9000, 12000, 15 000, or 18 000 mg melamine/kg. One male receiving the highest dose and one male receiving 6000 mg/kg died. Dose-related reduction in bodyweight gain was observed. The incidence of stones in the urinary bladder was dose-related and stones were found in all treatment groups among male rats. In the two highest female dose groups 25 % or more had stones. Ten animals of either sex from the 18 000 mg/kg, 6000 mg/kg and control group were evaluated by histopathology. Diffuse epithelial hyperplasia was found in the urinary bladder in 8/10 males and in 2/10 females in the high-dose group whereas in the low-dose group focal epithelial hyperplasia was observed in only 1/10 males and in none of the females (NTP, 1983).

In the second study the rats (10 of either sex) received feed containing 0, 750, 1500, 3000, 6000, or 12000 mg melamine/kg. Reduced body weight gain was observed in the two highest dose groups among males, but not among females. Dose-related increased incidence of stones in the urinary bladder was observed in male rats. None of the female rats had urinary bladder stones. Hyperplasia of the transitional epithelium of the bladder was present in a dose-related manner among males in the three highest dose groups, and only in males with urinary stones. This was accompanied by prominent capillaries and occasional oedema and scattered mast cells in the submucosa. The histopathology was later re-examined in a coded fashion and a higher incidence of hyperplasia of the bladder epithelium was found in most treatment groups compared with the original data (Melnick et al., 1984). In the female rats there were no evidence of urinary bladder stones or hyperplasia of the bladder epithelium, but dose-related calcerous deposits were observed in the straight segments of the proximal tubules. At day 65, urine from five rats of either sex in the lowest dose group and in the control group were examined clinically/chemically and microscopically. There were no differences attributed to the presence of melamine, and there were no evidence of melamine crystalluria.

The third 13 week study in rats was performed in order to investigate the effect of ammonium chloride on urinary stone formation in rats receiving 12000 mg melamine/kg feed. The addition of 1 % ammonium chloride to the drinking water had no effect on stone formation (NTP, 1983).

The CONTAM Panel converted melamine concentrations in food used in the first two NTP sub-chronic studies into daily intake (mg/kg b.w. per day) by use of information on mean weekly feed consumption and b.w. that was obtained from NTP (Appendix II). Feed consumption was quite stable whereas the b.w. increased, thus the calculated melamine intake decreased during the studies. Daily intake in the first week, mean during the studies and the last week are shown (Table 37). Since feed consumption and b.w. data were available, this approach was chosen instead of using standard conversion factors and/or standard feed consumption data as given by the International Programme on Chemical Safety (WHO/IPCS, 2008).

**Table 37:** Summary of data on selected studies of melamine-induced urolithiasis in male rats[1].

| Concentration in diet mg/kg | Intake at last week of the study mg/kg b.w. per day | Mean intake during the study mg/kg b.w. per day[2] | Intake at first week of the study mg/kg b.w. per day | Urinary bladder stones | Hyperplasia of the bladder epithelium | Reflux nephropathy[b] |
|---|---|---|---|---|---|---|
| NTP 1983, F344 rats, 14 days study | | | | | | |
| 0 | | | | 0 | | |
| 5000 | | | | 0 | | |
| 10 000 | | | | 4-5/5 | | |
| 15 000 | | | | 4-5/5 | | |
| 20 000 | | | | 4-5/5 | | |
| 30 000 | | | | 4-5/5 | | |
| Study I, NTP 1983, 13 weeks study[a] | | | | | | |
| 0 | 0 | 0 | 0 | 0/12 | 1/10 | 0/10 |
| 6000 | 460 | 578 | 1077 | 6/12 | 4/7 | 0/10 |
| 9000 | 719 | 854 | 1264 | 8/12 | | |
| 12 000 | 783 | 1134 | 1655 | 12/12 | | 6/9 |
| 15 000 | 1158 | 1395 | 1860 | 10/12 | | |
| 18 000 | 1482 | 1712 | 2400 | 12/12 | 10/10 | |
| Study II, NTP 1983, 13 weeks study[a] | | | | | | |
| 0 | 0 | 0 | 0 | 1/10 | 1/8 | 0/10 |
| 750 | 60 | 73 | 94 | 2/10 | 4/9 | |
| 1500 | 109 | 144 | 152 | 5/10 | 1/10 | |
| 3000 | 241 | 292 | 300 | 7/10 | 6/10 | |
| 6000 | 310 | 576 | 568 | 9/10 | 6/10 | 0/10 |
| 12 000 | 1043 | 1221 | 1343 | 9/9 | 8/9 | 6/9 |
| Ogasawara 1995, F344/DuCrj, 36 weeks study | | | | | | |
| 0 | 0 | | | 0/10 | | |
| 10000 | 349 | | | 7/19 | | |
| 30000 | 1028 | | | 6/20 | | |
| NTP 1983, 105 weeks study[a,c] | | | | | | |
| 0 | 0 | | | 0/45 | 0/45 | 1/49 |
| 2250 | 126 | | | 1/50 | 1/50 | 7/50 |
| 4500 | 263 | | | 10/49 | 2/49<br>1/49<br>papilloma<br>8/49<br>carcinoma | 19/49 |

[a] Histological data from Melnick et al., 1984; [b] Data from Hard et al., 2009; [c] Mean food consumption and body weight at the last week of the study; [1] Female animals were less susceptible and not included in the table; [2] Data used for BMD modelling.

Induction of bladder calculi by melamine has been investigated using male weanling F-344 rats that were given feed containing melamine at concentrations 0.2, 0.4, 0.7, 1.0, 1.3, 1.6, or 1.9 % (equal to 2000, 4000, 7000, 10000, 13000, 16000, and 19000 mg/kg feed) for 28-29 days. Urinary calculi were detected at dietary concentrations reaching from 4000 to 19000 mg/kg feed, showing a steep sigmoid dose response curve, with uroliths in 20 % of animals in the 7000 mg/kg group and in 70 % of the animals in the 10000 mg/kg group. Body weight gain was reduced in all the exposure groups from exposure day 2 to termination of the study. Water consumption increased with dose, and food consumption decreased in the two highest dosage groups. Urinary pH was slightly acidic (pH 6.7-7.1) compared to that of the controls (pH 7.5). Changes in urinary electrolyte concentrations were also observed, with lower concentration of Ca2+, Na2+, and Cl-, and increased concentration of Mg2+, K+, and NH4+, phosphate and sulphate. The calculi contained predominantly melamine and protein,

with traces of phosphate, oxalate, and uric acid. Hyperplasia was found in 94 bladders at dietary doses of 7 g/kg or more, and 93 of these were found to contain calculi by gross examination. The incidence of calculi found by histopathological examination was lower than by gross examination of the bladders, indicating that preparation for microscopic examination result in loss of stones. Further details from the study are not available since the original data are unpublished (American Cyanamid Company) and referred to in Heck and Tyl (1985).

Wistar rats (both sexes) fed with diet containing 1.5 % (15 000 mg/kg) melamine for 22-25 weeks and 36-40 weeks after weaning did not develop urolithiasis as observed by authopsy. The authors proposed crystal depots in the papillae that spontaneously dissolved after fixation as an explanation. However, in the group fed for 22-25 weeks, hyperplasia in the bladder was seen in 1/21 rats, hyperplasia in urether in 3/21 rats, hyperplasia in the urinary pelvis in 5/21 rats and dysplasia in the pelvis in 1/21 rats. No such alterations were seen in the 22 controls. Rats fed with melamine for 36-40 weeks had hyperplasia in the bladder in 1/20 animals, hyperplasia in urether in 2/20 rats, hyperplasia in the urinary pelvis in 1/20 rats and dysplasia in the pelvis in 2/20 rats, but not in any of the 36 controls (Cremonezzi et al., 2004).

**Mice**

One 13 week study has been conducted in B6C3F1 mice (10 of each sex). They received feed containing 0, 6000, 9000, 12000, 15000, or 18000 mg melamine/kg. One of the mice receiving feed containing 9000 mg/kg died. Body weight gain was depressed by 9 % or more in all dose groups. The incidence of mice with bladder stones was greater in males than in females, and the incidence increased from 12000 mg/kg onwards in a dose related manner (NTP, 1983).

BALB/c mice (both sexes) fed a diet containing 1.2 % melamine (12 000 mg/kg) for 18-22 weeks with and without 6 % lipids of different composition developed bladder stones in 60-85 % in all groups, except in the control group receiving no melamine and which had no stones. The group receiving melamine without extra lipids showed dysplasia or carcinoma in situ and/or hyperplasia in the bladder of 16/27 mice, dysplasia or carcinoma in situ and/or hyperplasia in the ureter in 10/27 mice and dysplasia or carcinoma in situ and/or hyperplasia in the renal pelvis of 2/27 mice. No lesions were seen in bladder and ureter in the 21 controls, but in the renal pelvis lesions were seen in two of the animals (Cremonezzi et al., 2004).

6.2.1.4. Long-term studies

**Rats**

50 male F344 rats were fed diets containing 0, 2250, or 4500 mg melamine/g feed, and 50 female F344 rats were fed diets containing 0, 4500, or 9000 mg melamine/g feed for 105 weeks, starting at 6 weeks of age. Feed consumption and body weight was unaffected by the presence of melamine. Survival of the high-dose group of male rats was significantly reduced compared with the control group. The urinary bladder was the primary site affected in male rats. There was a positive dose-related trend in transition cell carcinomas of the urinary bladder in male rats. The incidence of urinary bladder stones was significantly increased in the high-dose male rats (20 %) compared to controls (0 %). A significant association was found between the presence of bladder stones and bladder tumours. Chronic inflammation of the kidney was significantly increased in dosed female rats, but not in male rats (NTP, 1983).

A recent re-evaluation of the histopathological changes from the 13 weeks and 2 years rat NTP toxicity studies identified nephral changes extending from papilla to cortex, including tubular dilatation at 13 weeks, and fibrotic scars with tubule loss and collagen deposition after two years (Hard et al., 2009). The findings are summarised in Table 37. The authors used the term retrograde nephropathy to describe the lesions, and suggested that melamine precipitation in the lower urinary tract may have created pressure effects through transient obstruction leading to the renal changes.

In a study on the effect of NaCl on melamine induced urolithiasis and urinary bladder carcinogenesis, six weeks old F344/DuCrj male rats were fed diets containing 0, 1 % or 3 % of melamine, with or without 5 % or 10 % NaCl for 36 weeks (for 0-melamine, not 5 % NaCl), followed by a recovery period of four weeks. Calculus formation as well as transitional cell papillomas and carcinomas of the urinary bladder increased by increasing melamine concentration, but were dose-dependently suppressed by the simultaneous NaCl treatment. The NaCl effect was attributed to the increased urine production. The calculi consisted of an equimolar amount of melamine and uric acid. The authors concluded that the melamine induced proliferative lesions of the urinary tract in rats were due to irritation from the calculi, and not caused by melamine or melamine metabolites interacting with the bladder epithelium (Ogasawara et al., 1985).

**Mice**

Groups of 50 male or female B6C3F1 mice were fed diets containing 0, 4500, or 9000 mg melamine/g feed for 105 weeks from six weeks of age. Survival of the high-dose group of male mice was significantly reduced compared with controls. The incidence of urinary stones obtained by gross examination of the urinary bladder was increased markedly in treated males, with 4 % incidence in controls, 85 % incidence in the low dose group and 93 % in the high-dose group. The incidence was 8 % in high-dose females. In males, acute and chronic inflammation and epithelial hyperplasia of the urinary bladder was also markedly increased. No neoplastic or non-neoplastic lesions were associated with the administration of melamine in mice (NTP, 1983).

6.2.1.5. Genotoxicity and carcinogenicity

Melamine has been considered non-genotoxic in different in vitro and in vivo tests (WHO, 2009a). The International Agency for Research on Cancer (IARC) noted that the non-DNA reactive mechanism by which melamine produced urinary bladder tumours in male rats occurred only under conditions in which calculi were produced. Melamine was not classifiable as to its carcinogenicity to humans (group 3), but it was sufficient evidence in rats and mice for the carcinogenicity of melamine under conditions in which it produced bladder calculi (IARC, 1999). Melamine has induced proliferative epithelial lesions in the urinary tract sometimes in the absence of observed calculi, but this may be due to loss of calculi by the tissue preparation method (Melnick et al., 1984; WHO, 2009a).

6.2.1.6. Reproductive and developmental toxicity

There was no evidence of adverse effects on the reproductive organs by macroscopic and microscopic investigation of mammary glands, ovaries, uterus, testes, prostate and seminal vesicle (Melnick 1984, NTP, 1983). According to WHO (2009a) melamine is not teratogenic in the rat. The NOAEL was about 1060 mg/kg body weight per day for fetal toxicity and about 400 mg/kg per day for maternal toxicity. The foetal toxicity may have been mediated by the maternal toxicity (WHO, 2009a). No differences in foetal body weights and no malformations were seen in offspring from pregnant rats given intraperitoneal injections of 70 mg melamine/kg b.w. on days 4 and 5, 7 and 8 or 11 and 12 of gestation. There was no significant increase in resorptions in the animals dosed at day 4 and 5 as compared to control animals. However, data from concurrent control animals were not presented (Thiersch, 1957).

**Summary of toxicity of melamine in laboratory animals**

The kidney was the main target of melamine toxicity. Melamine precipitated in the nephral tubuli, renal pelvis and urinary bladder of mice and rats. As a consequence, hyperplasia and dysplasia of the bladder epithelium was formed. The pressure effects caused by constriction by precipitates in tubuli also lead to nephral dilatation and fibrotic scars extending from papilla to cortex. Male rats were more susceptible to melamine than female rats and mice. The CONTAM Panel concluded that the 13-weeks NTP studies with exposure of male rats provided the best basis for dose response modelling.

### 6.2.2. Cyanurate

6.2.2.1. Acute toxicity

The acute oral toxicity of cyanuric acid and sodium cyanurate is low and reported to be between 1500 mg/kg body weight and 10000 mg/kg body weight in rats and 3400 mg/kg in mice (OECD, 1999; WHO, 2004).

6.2.2.2. Short-term and sub-chronic studies

Mice (B6C3F1) were given sodium cyanurate in drinking water at concentrations up to 5375 mg/l, equivalent to 0, 252, 522 or 1500 mg/kg body weight per day for 13 weeks. The only compound-related changes reported were the occurrence of bladder calculi in males in the group given the highest dose. The NOEL reported was 1792 mg/l, equivalent to 522 mg/kg body weight per day (WHO, 2004).

Charles River rats were given drinking water containing sodium cyanurate at concentrations 896, 1792, or 5375 mg/l (equivalent to 72, 145, or 495 mg/kg body weight per day) for 13 weeks. In the group receiving the highest dose 7/28 males showed epithelial hyperplasia of the bladder. No treatment-related effects were seen in the kidneys or in any other tissue (WHO, 2004).

Weanling Wistar-derived rats (Rochester strain) were given sodium cyanurate in the feed at doses 0, 8000 mg/kg feed (10 males, 10 females) and 80 000 mg/kg feed (20 males, 20 females) for 20 weeks. Fourteen of the 20 males and 4/20 females at the highest dose died. At autopsy, organ weights were normal except for an increased kidney weight/body weight ratio in the high-dosed females. Histological examination of the kidneys showed dilation of distal collecting tubules and ducts of Bellini, with focal areas of epithelial proliferation in high-dosed animals (Hodge at al., 1965).

An oral toxicity study was performed in SD (Crj: CD) rats by an OECD combined repeat dose and reproductive/developmental toxicity screening test. Isocyanuric acid was administered by gavage at doses of 10, 40, 150 and 600 mg/kg per day for 45 days in males and from 14 days before mating to day 3 of lactation in females. Isocyanuric acid induced toxic effects at 600 mg/kg in both sexes. Depression of body weight gain was observed in males. Urinalyses of males revealed appearance of crystals, and increases of erythrocytes and leukocytes. Significant decrease in erythrocyte counts, haemoglobin concentrations and haematocrit values were observed. Increases in blood urea nitrogen and creatinine, and a decrease of sodium were seen. In histopathological examination, dilatation of the renal tubules, necrosis or hyperplasia of the tubular epithelium, increased basophilic tubules, neutrophilic infiltration, mineralization and fibrosis in the kidney, hyperplasia of the mucosal epithelium in the urinary bladder and vacuolization of the zona fasciculata in the adrenals were observed in both sexes. Absolute and relative kidney weights and relative adrenal weights were increased in both sexes (OECD, 1999).

6.2.2.3. Long-term studies

Charles River CD rats (80 of each sex) were given sodium isocyanurate in drinking water at concentrations of 0, 400, 1200, 2400 or 5375 mg/l (equivalent to 0, 26, 77, 154 or 371mg/kg body weight per day) for 2 years. A second control group received sodium hippurate, resulting in the same amount of sodium as the highest treatment group. 10 animals were killed at 6, 12 and 18 months. Lesions to the urinary tract and heart were reported in males at the high dose. Nine of the 11 males with heart lesions receiving a dose of 371 mg/kg body weight per day that died or were killed in the first year of the study also showed calculi in the bladder. Calculi were not found in all these animals but an expert Panel observed fragments of calculi in a number of histological slides, suggesting that the calculi may have been lost in fixation. The authors considered that the urinary lesions (including hyperplasia, bleeding and inflammation of the urinary bladder epithelium, inflamed urethers and rental tubular nephrosis) were probably related to calculi, and the heart lesions were secondary to uremia

secondary to the urinary tract lesions. Urolithiasis was more frequent in males than females at the high dose. The NOEL was 154 mg/kg body weight per day (WHO, 2004).

In a two-year study in which mice (B6C3F1, 100 of each sex) received doses of sodium cyanurate at 0, 400, 1200, 2400, and 5375 mg/l (equivalent to 0, 72, 30, 110, 340, 1523 mg/kg body weight per day) there were no treatment related changes in the survival, incidence of tumors or histopathological changes. (WHO, 2004).

Two groups of 3 young adult beagle dogs were maintained on diets containing 0.8 and 8 % sodium cyanurate. After 6 months, dogs given the diet containing 0.8 % cyanurate were sacrificed. Kidney tissues were normal. The three dogs on the 8 % cyanurate diet were fed on this diet for 2 years. One dog died after 16 months, a second after 21 months. Causes of death were not clearly established. The third dog was sacrificed after 24 months and gross evidence of kidney fibrosis was noted. According to the authors, it seemed reasonable to suggest that continuous stimulation of the excretory portions of the nephron led to hypertrophy, atrophy and fibrosis (Hodge et al., 1965).

#### 6.2.2.4. Developmental and reproductive toxicity

Potential developmental toxicity has been evaluated in rats and rabbits. Pregnant Dutch belted rabbits were gavage-fed 50, 200, and 500 mg sodium cyanurate/kg b.w. during days 6-18 of gestation. No evidence of a dose related increase in the incidence of malformations or skeletal anomalies in the foetuses was observed (FMC Corporation, unpublished results referred to in Hammond et al., 1986). Pregnant CD rats were gavage-fed sodium cyanurate at 200, 1000 and 5000 mg/kg per day during days 6-15 of gestation. Controls received sodium hippurate (control for sodium exposure) or tap water. No evidence of cyanurate related foetotoxicity or teratogenicity was found (Cascieri et al., 1983). Sodium cyanurate was administered to rats in the drinking water at concentrations 400, 1200, and 5375 mg/l throughout three consecutive generations. Two control groups received either tap water or sodium hippurate. No compound related mortality or adverse reactions connected to reproductive performance were observed during the study. Concerning pathologic changes, the only effect observed was that in a few high cyanurate-dosed males there were calculi in the urinary bladder, together with microscopic evidence of epithelial hyperplasia or chronic cystitis. The histological changes were attributed to the chronic irritation by the calculi (Wheeler et al., 1985; Hammond et al., 1986; WHO, 2004).

#### 6.2.2.5. Genotoxicity

Cyanuric acid has been considered non-genotoxic in an adequate battery of *in vitro* and *in vivo* tests (reviewed by Hammond et al., 1986) (WHO, 2004).

### 6.2.3. Ammelide and ammeline

Female Sprague Dawley rats (6 animals per group, 240-285g) were gavage-fed a single dose of 0, 10, 13, or 100 mg/kg b.w. of ammeline or ammelide as a suspension in 1 % carboxymethylcellulose. No toxicity with respect to renal function, kidney weight or histopathology of the kidneys was observed (Dobson et al., 2008).

### 6.2.4. Combined exposure to melamine and structural analogues

Female Sprague Dawley rats (10 animals per group, b.w. 24-285 g) were gavage-fed a mixture of melamine/cyanuric acid 400/400 mg/kg b.w. per day or a mixture of melamine/ammeline/ammelide/cyanuric acid, 400/40/40/40 mg/kg b.w.per day. Both mixtures were suspensions in 1 % carboxymethylcellulose, and given daily for three consecutive days. In addition, one group of animals received the melamine/cyanuric acid as a single dose. Both mixtures produced toxicity, and several animals were oliguric by the end of the three days dosing, particularly in the

melamine/cyanuric acid dosing group. There was a marked increase in blood urea nitrogen level and decreased creatinine clearance in all dosed groups. Urinary pH was also significantly decreased in all three 24-h urine samples. The kidneys from dosed animals were edematous and brownish-yellowish precipitate was seen in the tubules. Histological examination of frozen sections revealed that crystals were present in a large proportion of renal tubules, particularly in the medulla. They appeared to be sufficiently abundant to block tubular flow. Extensive tubular dilatation in distal tubules and basophilic debris in distal tubules and loop of Henle was found in sections from formalin-fixed tissue. This was most severe in the melamine/cyanuric acid group. The crystalline material in frozen kidney sections from a cat exposed to tainted food appeared to be identical to that in the treated rats. Infrared spectra from the precipitate in rat, cat and crystals from tainted wheat gluten matched the melamine-cyanuric acid cocrystal reference material. The concentration of melamine and cyanuric acid in the rat kidney tissue was 2000-3000 mg/kg wet tissue. Ammelide was present at measurable concentration in kidney tissue from all groups, which was explained by impurities of 0.2-0.4 % ammeline and 0.4-1.8 % ammelide in dosed compounds (Dobson et al., 2008).

In a metabonomic study of melamine-induced renal toxicity in rats, five groups of seven male Wistar rats were gavage-fed melamine at 600, 300, and 100 mg/kg b.w., cyanuric acid at 100 mg/kg b.w., and mixture of melamine and cyanuric acid (50 mg/kg b.w. each) for 15 days. The kidneys of the treatment groups were found to be oedematous with organ weight higher than those in the control group. Crystals were found near the papilla of the renal tubule in all treatment groups. In the 600 mg/kg melamine group and the melamine-cyanuric acid group, extensive tubular dilatation in distal tubules and a small amount of haemorrhage was shown by histological examination. The combined experimental results of metabolomics, serum biochemical analysis and histology indicated that melamine at all doses induced significant renal toxicity in a dose-dependent manner, and that the mixture of melamine and cyanuric acid (50 mg + 50 mg/kg b.w.) and 600 mg of melamine/kg b.w. resulted in the greatest renal toxicity and physiological alteration. The high-dose melamine (600 mg/kg b.w.) and low-dose melamine-cyanuric acid, both perturbed the urinary expression of different metabolites, but impacted the same metabolic pathways, including tryptophan, polyamine, and tyrosine metabolism and alteration in the structure of gut microflora (Xie et al., 2009).

Sprague Dawley rats (five of each sex) were fed with melamine contaminated pet food (ratio of melamine: cyanuric acid 6.8:1) mixed into the chow at 10 %, 20 %, 50 %, and 50 %-100 % (weight/weight) for 12 weeks. The 50-100% group received chow with 50-100 % contaminated feed the first 8 weeks and then 100 % contaminated feed for 4 weeks (Chen, 2009a). The mean dietary intake is shown in Table 38.

**Table 38:** Daily intake (mg/kg b.w.) of cyanuric acid and melamine in rats fed various concentration of adulterated chow (based on Chen, 2009a).

| | | Fraction of adulterated feed mixed into diet | | | |
|---|---|---|---|---|---|
| | | **10 %** | **20 %** | **50 %** | **50-100 %** |
| **Male** | **Cyanuric acid** | 6.0 | 11.8 | 29.4 | 38.3 |
| | **Melamine** | 40.6 | 80.4 | 200.4 | 260.9 |
| **Female** | **Cyanuric acid** | 7.2 | 14.6 | 35.2 | 60.3 |
| | **Melamine** | 48.9 | 99.7 | 240.0 | 410.7 |

One female and one male in the 50 % group died in the weeks five and nine, respectively. Increase in blood urea nitrogen, creatinine, and total cholesterol was seen in the highest dose group in both sexes, and increased phosphorous gamma-glutamyltransferase, triglyceride and creatine kinase was seen in males in the highest dose group. Decreased uric creatinine and bilirubin, as well as increased urine volume, was seen in both sexes in the highest-dosed group. Among males in this group also uric ketone concentration, pH and protein was decreased. Kidney weight was increased in both sexes in the high dosage groups. Changes in the kidney size and morphology were found variously in the two highest dosage groups. Melamine-cyanurate crystals were easily observed using a polarized microscope. Also slight to severe inflammatory cell infiltration, tubular dilatation and interstitial fibrosis was seen. Immunohistochemical markers related to renal injury and cell proliferation

(osteopontin and proliferative cellular nuclear antigen) increased slightly in the 50 % group and were substantially elevated in the highest dose group (Chen, 2009a).

Male rats (Sprague –Dawley, 5-6 months old) were orally exposed to melamine and cyanuric acid (1:1) at 0, 25, 50, or 100 mg/kg b.w. per day for 3 days (n=10/group, 9 at 100 mg/kg). The two highest doses led to increased blood urea nitrogen and creatinine, reduced creatinine clearance and enlarged kidneys. At 50 mg/kg b.w. crystals were mainly located in kidney medulla, while at 100 mg/kg b.w. crystals were seen throughout both cortex and medulla (Kim et al., 2010). Male rats (Sprague – Dawley, 10 weeks old, n=18/group) were exposed to melamine by a stomach tube at 2.4, 24 or 240 mg/kg b.w.per day, or to the combination of melamine and cyanuric acid at 1.2, 12, or 120 mg/kg per day, or to water (controls) for maximally 14 days. Blood and 24-h urine samples and kidney sections were evaluated on days 3, 7, and 14. Rats receiving the highest dose of melamine/cyanuric acid died from acute renal failure before day 7. In rats given 12 mg/kg per day of melamine/cyanuric acid, crystals were seen in proximal tubules in the cortex after 3 days, and the number increased across day 3, 7 and 14. Crystals were also present in distal tubules and collecting duct. Serum creatinine was increased from day 3 in this group (Kobayashi et al., 2010).

In a study so far published only in abstract form, nephrotoxicity and melamine-cyanurate-like crystals were observed in male and female F344 rats given melamine and cyanurate each at 33 mg/kg b.w. per day via the diet for 7 days. No effects were seen at 10 mg/kg b.w. per day melamine and 10 mg/kg b.w. per day cyanurate combined, or when melamine and cyanurate were administered individually at 200 mg/kg b.w. per day (Gamboa da Costa et al., 2010).

**Summary of combined effects of melamine and cyanurate in laboratory animals**

Several studies indicate that concomitant exposure to cyanuric acid at the same dose as melamine result in more than 2-fold increase in kidney toxicity. The metabolomical and histological study by Xie et al. (2009) and the abstract of Gamboa da Costa et al. (2010) suggest that a combination of melamine and cyanuric acid produced similar toxicity to a 12-20-fold higher dose of melamine administered alone.

## 6.3. Adverse effects of melamine and its analogues in livestock, fish and pets

Reports describing adverse effects of melamine and its analogues in production animals are limited. Therefore, the toxicity data of melamine, cyanuric acid, ammeline and ammelide are summarised together below.

### 6.3.1. Ruminants

Protein is the first limiting nutrient for ruminants grazing forages, but a high concentration of soluble proteins also adversely affects rumen fermentation and hence weight gain and milk production. Hence a major objective in ruminant nutrition is the finding of compounds that are convertible to protein by the forestomach flora, leading to intensive research into the so-called NPN (non-protein nitrogen) class of feed ingredients. For this purpose nitrogenous compounds were tested, as ingredients of livestock feeds, including melamine, cyanuric acid, as well as ammelide and ammeline, which were studied in sheep (Clark, 1966; MacKenzie, 1965; MacKenzie, 1966; MacKenzie and vanRensburg, 1968).

#### 6.3.1.1. Cattle

The use of melamine as an efficient N source for ruminants has been investigated by Newton et al. (1978). One mature steer was given 45 g per day melamine during 88 days in the diet. The melamine consumed amounted to an average of 14 g nitrogen per day. No data are detailed regarding the weight of the animal and no adverse effects were reported.

In a recent feeding trial, cows were fed with melamine-containing feed to investigate the carry-over of melamine residues in milk (see 6.1.1.4.1). Dairy cows weighing around 638 kg were fed with 17.1 g melamine per day and per cow (corresponding to approx. 27 mg/kg b.w.) during 8 days. No adverse effects were seen in the animals during the experiment, and milk composition remained unchanged (Cruywagen et al., 2009).

6.3.1.2. Sheep

Clark (1966) studied the value of melamine, as a non-protein nitrogen supplement, in Merino sheep. A first experiment was undertaken with adding melamine through permanent ruminen fistulae. One sheep (46 kg) was given 100 g (single dose), another animal (37 kg) was dosed with 50 g on 6 consecutive days, a third one (49 kg) was dosed with 25 g on 18 consecutive days. Three other sheep were dosed with 10 g melamine per day for 16, 31 or 39 days respectively. In a second experiment, several groups of three sheep were given melamine (7 g sheep per day) mixed in maize meal. Post-mortem investigation revealed crystals in the kidney tubules of all animals that died spontaneously or were sacrificed at the end of the exposure period. Only the sheep fed with 7 g melamine mixed in the feed survived the experiment and none of them showed any adverse effect. Melamine was found in the urine by addition of picric acid (picrate test), this reaction was considered a good presumptive test for melamine. As a conclusion of his study, Clark (1966) reported that an intake of > 10 g per day (> 250 mg/kg b.w.) resulted in crystalluria and consequent death in two sheep out of three (respectively after 16 and 31 days), whereas 7 g per day fed, during 6 weeks, had no adverse effects. The weights of the animals receiving 7 g melamine are not detailed. The author noticed that the animals refused to consume the total amount of maize containing melamine.

MacKenzie (1966) performed a toxicity test and a feeding trial with melamine in sheep. During the feeding test, two groups of 9 sheep with an average initial weight of 39 kg received melamine as a supplement in feed (10 and 20 g, corresponding to 256 and 513 mg/kg b.w. respectively). The trial was stopped after 28 days because the sheep showed significant weight losses and several were in critical condition. After that, the sheep returned to a diet without melamine. However, the author reported that sheep died in the group that received 10 g melamine without showing particular symptoms of physiological disturbance. No detailed post-mortem examinations were carried out.

Cyanuric acid was fed to sheep at 4 levels (0, 8, 16, 24 g per head per day) with maize meal as a daily supplement on a diet of low quality hay (Mackenzie, 1965). The test ran for 77 days and the average initial weight of the animals was 40.5 kg. The results indicated that the daily amount of cyanuric acid required to maintain life weight was between 8 g (198 mg/kg b.w.) and 16 g (400 mg/kg b.w.) per head. The sheep receiving 24 g (600 mg/kg b.w.) increased in weight but at this level of feeding the efficiency of cyanuric acid utilisation tended to decrease.

Cyanuric acid was considered as a better feed supplement to improve nitrogen balance than urea and was declared to be a good non toxic nitrogen supplement in sheep by Altona and MacKenzie (1964). These authors suggested that the lack of toxicity of cyanuric acid is due to a slow rate of hydrolysis by bacteria (Altona and MacKenzie, 1964; Clark et al., 1965).

In feeding trials, ammelide and ammeline were tested as sources of NPN for German Merino ewes (Mackenzie and vanRensburg, 1968). In the feeding trial, five groups of ten sheep with an average weight of 38 kg were fed once daily and during 42 days with supplements consisting of a mixture of maize meal and NPN compounds. Ammelide (139 g per group) or ammeline (114 g per group) alone as well as mixtures of both these substances (total amounts of ammelide and ammeline, of 126 g in a first mixture or 130 g in a second mixture) were added daily to the diet. After four weeks, the sheep receiving supplements containing ammeline began showing symptoms similar to those observed in sheep fed with diets containing melamine (inappetence, emaciation, excessive urination) in a previous study (MacKenzie, 1966).

An average daily intake of 296 mg ammeline/kg b.w. per day and 97 mg/kg b.w. of a mixture of ammeline and ammelide in the diet was sufficient to cause the death of half the sheep in the group.

Half of the sheep fed with mixtures of ammeline and ammelide died within 58 days of experiment. During the study, two sheep were killed for postmortem examination. The kidneys were enlarged and showed crystal formation. Results indicated that ammelide was not acutely toxic and did not cause toxicity when fed to sheep for a period of 6 weeks. Ammeline resulted in the development of a crystalluria syndrom. The authors concluded that their results indicate that neither ammelide nor ammeline were suitable sources of NPN in sheep. The authors also noticed that neither the ammeline nor the ammelide used were in pure form.

### 6.3.2. Pigs

Nephrotoxicosis, was observed by Gonzalez et al. (2009) in piglets consuming contaminated feed. Between November 2003 and September 2003, 300 to 400 45-60-day old Iberian piglets developed anorexia, polydipsia and lethargy. Piglets were from 5 different farms in Spain. Morbidity was between 40 % to 60 % and mortality ranged from 20 % to 40 % of the total population of postweaning piglets. Postmortem examinations were conducted in 9 piglets. Kidneys were enlarged with yellow foci in the cortex and medulla. Microscopically, these foci were accumulations of crystals within the lumina of dilated distal tubules and collecting ducts, causing flattening of the renal tubular epithelial cells. The crystals displayed a multicolour birefringence under cross-polarized light. The multinucleated giant cells surrounding the crystals, interstitial fibrosis and non suppurative infiltrates indicated a chronic inflammatory response. The diagnosis of toxicosis was based on the presence of characteristic crystals in tubules and ducts and the measurements of ammelide, ammeline, cyanuric acid and melamine in the affected kidneys from 4 piglets. Ammelide concentrations were highest, ranging from 39 to 92 mg/kg, followed by ammeline (from 20 to 34 mg/kg), melamine (from 9 to 92 mg/kg) and cyanuric acid (from 2 to 9 mg/kg). No data were available with regard to the levels of those compounds in the animal feed.

Renal failure in nursery pigs (6-9 weeks) due to melamine contaminated feed (> 4000 mg/kg) was also reported by Akrimajirachoote et al. (2008). These animals came from a 3000 sow farm in Ratchaburi province, in Thaïland, with 100 % morbidity rate and 70 % mortality rate. All the pigs were emaciated, dehydrated and had subcutaneous hemorrhages and swollen joints. 3 dead pigs and 6 blood samples were subjected to necropsy investigation. Necropsy findings showed congestion and infarction and presence of yellowisch crystal in the kidneys. Histopathological examination showed enlargement and inflammation as well as accumulation of brownish crystals in the renal tubules.

Experimental feeding of melamine and cyanuric acid in pigs was performed by Reimschuessel et al. (2008). Four pigs were fed a target dosage of melamine (400 mg/kg b.w.), cyanuric acid (400 mg/kg b.w.) or melamine and cyanuric acid (400 mg/kg b.w. for each compound) daily for 3 days. Animals were euthanatized 1, 3, 6, 10 or 14 days after administration ceased. Fresh, frozen and formalin-fixed kidneys were examined for crystals. In this study, renal crystals were experimentally induced in pigs via co-administration of a 1: 1 ratio of melamine and cyanuric acid. However, administration of melamine or cyanuric acid alone generally did not result in crystal development. All animals fed the combination of melamine and cyanuric acid developed goldbrown renal crystals arranged in radial spheres. Spectral analyses of crystals were consistent with melamine-cyanurate complex crystals.

### 6.3.3. Rabbits

No data were available in rabbits.

### 6.3.4. Poultry

No data were available in poultry.

### 6.3.5. Fish

Experimental feeding of melamine and cyanuric acid in fish was performed by Reimschuessel et al. (2009) in a number of studies.

Reimschuessel et al. (2009) reported the results of the determination of melamine and cyanuric acid residues in catfish and trout filets. A total of 20 fish were harvested at 1, 3, 7, 14, 28 and 42 days after a single oral dose of 20 mg/kg b.w. of melamine, a single dose of 20 mg/kg b.w. of cyanuric acid, or a single dose of 20 mg/kg b.w. of melamine and 20 mg/kg b.w. of cyanuric acid together. Renal crystals formed in fish given both melamine and cyanuric acid, persisting for weeks after the single dose.

A total of 75 fish from several species (21 tilapia, 24 rainbow trout, 15 channel catfish and 15 Atlantic salmon) were fed with melamine and / or cyanuric acid by Reimschuessel et al. (2009). A target dosage of melamine (400 mg/kg b.w.), cyanuric acid (400 mg/kg b.w.) or melamine and cyanuric acid (400 mg of each compound/kg b.w.) was given daily for 3 days and fish were euthanatized 1, 3, 6, 10 or 14 days after administration ceased. Fresh, frozen and formalin-fixed kidneys were examined for crystals. In this study, renal crystals were experimentally induced in fish via coadministration of a 1:1 ratio (ranging for each compound from 390 to 452 mg/kg b.w. for trout, from 376 to 422 mg/kg b.w. for salmon, from 418 to 479 mg/kg b.w. for catfish) of melamine and cyanuric acid. For tilapia, capsules were filled with 150 mg of melamine, 150 mg of cyanuric acid or 75 mg of each compound. Doses in tilapia ranged from 300 to 456 mg/kg b.w. for melamine, 147 to 390 mg/kg b.w. for cyanuric acid and from 39 to 114 mg/kg b.w. for the combination. However, administration of melamine or cyanuric acid alone generally did not result in crystal development. Renal crystals formed in fish that received both compounds administered at the same time and also in fish that received melamine first, then cyanuric acid several days later. Crystals in fish kidneys were distributed throughout the renal tubules and collecting duct system.

In another study, one group of tilapia was given lower doses of melamine and cyanuric acid (approximatively 3 to 17 mg/kg b.w). The aim of the study was to establish a lower limit for biological effects. Fish were dosed daily for 3 days and then euthanised 1-14 days after dosing. Three tilapias consumed 3 - 4 mg/kg body weight each of melamine and cyanuric acid on each of 1, 2 or 3 days, respectively, and were euthanased 1 day after dosing. None of these fish developed renal crystals. Measurable quantities of melamine were found in the tissues (0.37 - 0.84 mg/kg). Crystals were found in the kidneys of tilapia that received more than 7 mg/kg body weight of melamine and cyanuric acid on each of 3 days (Andersen et al., 2009).

Fish have mesonephric kidneys. They excrete most of their nitrogenous waste via the gills and can endure more extensive renal damage than most mammals. In the studies performed by Reimschuessel, most of the fish survived the renal damage induced by the melamine-cyanurate complex crystals. However, even fish died from major renal damage, death being caused by a mechanism similar to acute uric acid nephropathy in humans (Reimschuessel et al., 2009).

Shrimp specimens from rearing facilities in Indonesia and India were found to present prominent golden to greenish-brown needle- and plate-like birefringent crystals within multifocal hemocytic granulomas in the antennal gland tubules and peritubular hemal sinuses. The crystals' appearance was very similar to melamine-cyanuric acid-induced crystals described from cat and dogs kidneys with melamine-associated renal failure. Melamine was detected in feed samples at levels of 183.39 and 112.50 mg/kg. A bioassay was performed feeding shrimps with the melamine-contaminated feed. Prominent granulomas in the antennal gland with the characteristic crystals were induced within 10 days of the first feeding, experimentally confirming the direct relationship of melamine-adulterated feed to the pathology observed (Lightner et al., 2009).

### 6.3.6. Frogs

Poisoning cases were described in fattening frog farms in the Nakorn Pathum and Chumporn provinces, in Thaïland, in 2007. According to Wongthai et al. (2008), the death was mainly attributed

to renal failure. The conditions were observed in 6 farms with earth and concrete pond culture systems. The affected frogs gradually died within 2 months without any significant signs. Frogs even exhibited normal appetite. Animals were examined by the necropsy services. Postmortem examinations revealed the presence of serosanguineous fluid in the abdomen, haemorrhage and necrotic areas in the liver and kidney, nephromegaly and fibrin inflammation in the pericardium, kidney and liver. Histopathological signs included brown crystal-like melamine accumulation and degeneration in the renal tubules. Concentrations of melamine in feed or in tissues were not reported by this incident.

### 6.3.7. Pets (cats and dogs)

Melamine has been reported to cause diuresis and crystalluria in dogs. A combination of melamine and cyanuric acid has caused deaths in cats and dogs, inducing crystalluria, uroliths and nephrotoxicity (Brown et al., 2007; Canciolo et al., 2008; Thomson et al., 2008; Osborne et al., 2009). These studies, based mainly on histomorphometry and histochemistry, describe the research that was conducted following the discovery that dogs and cats had become ill after eating certain food lots of wet pet food in North America in 2007. These lots of pet food had been formulated with wheat gluten originating from China, which is added to wet pet foods as a thickening agent. In summary, the consumption of melamine and cyanuric acid resulted in extensive lesions in renal tubular epithelial cells associated with numerous characteristic crystals in the renal tubular lumens. The severity of renal dysfunction seemed to be influenced by several factors, including the age of the animals, the quantity of adulterated food consumed, the pH of the stomach and the kidneys, and the status of the renal health. However information on the doses of melamine and cyanuric acid resulting in illness and death is not available.

In a clinical study (Puschner et al., 2007), melamine and cyanuric acid were added alone or in combination to the diet of 4 individual cats. In the first part of the experiment, two cats received a diet for a period of 11 days to which melamine was added alone in a concentration of 0.5 %. Based on feed consumption, the actual dose varied between 44 – 121mg/kg b.w.per day in the animal receiving a diet with 0.5 % melamine and remained constant at 181 mg/kg b.w. per day in the animal that received 1 % melamine in the diet. No adverse effects were noted in either cat.

In an additional study (part 3 in the publication) one cat was given a diet with cyanuric acid alone in increasing concentrations of 0.2 % (49 mg/kg b.w.) for 4 days, 0.5 % (121 mg/kg b.w.) for 3 days, and 1 % (243 mg/kg b.w.) for 3 days. Again, there was no evidence of renal failure as measured by serum creatinine and urea nitrogen, and no gross or histological abnormalities were found at post mortem inspection.

The apparent low toxicity of melamine and cyanuric acid when given alone was in sharp contrast to the findings obtained when melamine and cyanuric acid were applied simultaneously. When cats received a combination with 0.2 % melamine and 0.2 % cyanuric acid (cat 2), 0.5 % melamine and 0.5 % cyanuric acid (cat 3), and 1 % melamine and 1 % cyanuric acid (cat 4), within 12 h after the first dosing, all 3 cats developed slight depression, vomiting and anorexia. The actual intake from the 1$^{st}$ meal varied between 32 mg/kg b.w. melamine and 32 mg/kg cyanuric acid (0.2 % diet) to 181 mg/kg b.w. melamine and 181 mg/kg cyanuric acid (1 % diet). Subsequently the cats did not eat or only partially ate the second meal, resulting in an estimated dose of 2 mg/kg b.w. and 2 mg/kg cyanuric acid in the 0.2 % diet, and 54 mg/kg b.w. melamine and 54 mg/kg b.w. in the 1 % diet at the second day of the experiment. Renal function was significantly impaired in all 3 cats when assessed 36 hrs after the first exposure. In all 3 animals, amorphous, rounded and fan-shaped crystals were found in the urine as well as in the renal tubules, particularly the collecting ducts and the distal tubules, at post-mortem inspection. In the animal receiving the highest dose (the diet with 1 % melamine and 1 % cyanuric acid) on average 50 crystals were found per 1 cm diameter region of a 5 micrometer section within the cortex. Subsequently, the renal interstitium was oedematous, and tubular architecture and the epithelium were affected throughout the nephron. At the cortico-medullary junction, in the region of the distal straight tubules, the interstitium was expanded by oedema and hemorrhage. In the cats

receiving the combination at a concentration of 0.5 % and 1 % melamine and cyanuric acid, eosinophilic granula and hyaline protein casts were present in the lumina of the tubules, and the arcuate and interlobar vessels and vasa recta were congested.

Chemical analysis (LC/MS) of the kidney tissue revealed dose-dependent melamine concentrations of 496 - 734 mg/kg wet weight and cyanuric acid concentrations of 487 - 690 mg/kg wet weight. The analytical method included dissolution of melamine-cyanurate crystals present in the renal tissue.

In the animal dosed only with cyanuric acid, a concentration of 22 mg/kg wet weight was found in the renal tissue. Tissues levels of melamine form the first part of the experiment (exposure to melamine alone) are not presented. In none of the animals exposed to the combination of melamine and cyanuric acid, ammelide or ammeline was detected in renal tissue including the precipitated crystals.

The pathological findings reported in this study are very similar to those reported in cats (and dogs) following ingestion of contaminated pet food (Brown et al., 2007). Hence, even considering the fact that for the different dosing regimes only 1 cat was used in this study, the presented results allow the following conclusions:

- in a cat, dietary short term exposure (10 days) to melamine alone did not induce signs of renal function impairment up to a daily dose of 181 mg/kg b.w. per day;

- dietary short term exposure (3 days) to cyanuric acid alone did not induce signs of renal function impairment up to a daily dose of 243 mg/kg b.w. per day;

- dietary exposure to a 1:1 combination of melamine and cyanuric acid induced, at doses below 32 mg/kg b.w. of each compound, clinical symptoms such as vomiting and feed refusal within 12 hrs. Post mortem inspection showed multiple crystal formation in renal tubules, interstitial oedema and hemorrhages, and subsequent impairment of renal structure and obstructive renal failure (Puschner et al., 2007).

In summary, the consumption of melamine and cyanuric acid resulted in extensive lesions in renal tubular epithelial cells associated with numerous characteristic crystals in the renal tubular lumens. The severity of renal dysfunction seemed to be influenced by several factors, including the age of the animals, the quantity of adulterated food consumed, the pH of the stomach and the kidneys, and the status of the renal health.

### 6.4. Human data

More than 20 million children in China have been examined, and by the end of November 2008, 294 000 young children and infants had been diagnosed to have urinary tract stone caused by consumption of milk or milk products adulterated with melamine. Most patients had no symptoms and signs, but acute renal failure occurred in a small proportion of the patients. More than 50000 infants have been hospitalized, and six deaths have been confirmed to be related to melamine exposure (Chen, 2009b).

The report from the WHO expert meeting in 2008 (WHO, 2009b) referred to several unpublished studies and reports, and a number of these are now available. Below, published reports after the melamine incidence in China are summarised and the summary data is illustrated in Table 39.

In a cross-sectional study from Peking, China, the primary outcome was the presence of urinary stones and the secondary outcomes were clinical manifestations and laboratory abnormalities associated with melamine exposure. All children 26 months of age or younger who were brought to the Peking University First Hospital for screening for urinary stones between September 17 and October 3, 2008 were studied. Of the 589 children that were screened 341 were boys and 248 girls. History of exposure to contaminated formula, including brand name, duration of exposure and eventual combination with breast-feeding was obtained by questionnaires. Based on the amount of melamine

contamination the formulas were grouped into three categories: high melamine formula (> 500 mg/kg), moderate melamine formula (<150 mg/kg) and no melamine formula. Content of individual formula (63 brands/sub-brands from 22 companies, children in the study were exposed to formula from 7 of the companies) were listed. Children had to have been fed formula for at least 30 days to be considered to have been exposed. Findings with regards to urinary stones by ultrasonography were categorized into definite stones, suspected stones, or no stones. Stones were found in 50 of the children, including in 8 who had not received melamine-contaminated formula, 112 were suspected to have urinary stones and 427 had no stones. Those exposed to high melamine content of infant formula were 7 times as likely to develop stones as non-exposed children. Children with moderate exposure were twice as likely to develop stones as non-exposed children. Most of the melamine associated stones were not characterized by shadowing on ultrasonography, and this distinguishes them from typical urinary tract stones. Most children with melamine associated urolithiasis had non-specific symptoms and urinary findings. The incidence of haematuria, leukocyturia, and proteinuria did not differ significantly among the groups. The incidence of glomerular dysfunction, measured by elevation of urinary microalbumin levels and/or transferrin levels among children suspected to have stones was significantly increased compared with children who did not have stones. Age, sex, and use of formula alone or in combination with breast milk were not significantly associated with the presence or absence of stones. Preterm birth or high melamine content in the formula received was significantly associated with the presence of stones. Children exposed to formula with high melamine content were also more likely to have suspected stones (Guan et al., 2009).

A further consideration on the mechanism of melamine containing urinary tract stone formation in children has been published by Lam et al. (2009). Among many factors studied (e.g. hyper calciuria, hyperphosphaturia, hyperoxaluria) apart from urinary melamine, pH was the only factor showing a significantly difference between the cases (children with stones; n=14; pH range 4.5 - 7) and controls (children without stones; n=20; pH range 5.5-7). Seven (50 %) of the cases had a urinary pH < 5.8 while in the group of controls only 4 (25 %) had a urinary pH < 5.8. In > 30 % of the cases hyperuraturia was observed, while in none of the controls this condition was seen. Urinary melamine concentrations ranges from 0.08 to 37 (median 6.6) μg/mmol creatinine in the controls and from 0.87 to 2002 (median 21) μg/mmol creatinine in the patients with stones. No difference was found in urinary cyanuric acid concentration between cases and controls. A correlation between renal stone size and urinary melamine concentration was reported: for stones with a diameter < 10 mm, a 10 μg/mmol creatinine increase in urinary melamine concentration was associated with approximately 1 mm increase in stone diameter. Within this group of individuals, a urinary melamine concentration of 7.1 μg/mmol creatinine was suggested to predict a significant risk of urinary stone formation with a sensitivity of 87 % and a specificity of 60 %.

The Panel noted that urinary samples were only collected and analysed 10 days after cessation of exposure to melamine-containing infant food. Given the rapid elimination of melamine it is rather surprising that melamine could be detected in the urine at all in one case even at a concentration of 2 mg/mmol creatinine. Also cyanuric acid was found, but in concentrations not related to the concentration of melamine. These concentrations of melamine and cyanuric acid might be caused by dissolution of urinary tract precipitates, but the authors also mention that in the controls melamine could be found in the urine, though at much lower concentrations. The analyses were carried out by GS-MS after solid phase sample clean-up.

A case-control study from Taiwan was conducted in children (n=1222, 682 boys) aged 0 to 16 years with potential exposure to contaminated dairy products. Preterm children, children with congenital abnormalities of the genitourinary tract or with chronic diseases were excluded. Cases (n= 14, 7 boys) were defined as presence of calculi in the kidney or collecting system (nephrolithiasis) by renal ultrasonography. Based on melamine concentration in consumed contaminated milk products (>2.5 mg/kg, 0.05-2.5 mg/kg, <0.05 mg/kg (detection limit)) subjects were classified into high exposure, low exposure, and control group. Blood pressure, urinalysis, urine calcium and creatinine, renal function tests, and renal ultrasonography were evaluated. Only 2/10 nephrolithiasis patients had elevated urine melamine levels, and this was not considered as a sensitive test. Two non-

nephrolithiasis controls from the study population were matched to each nephrolithiasis case by age and sex. The duration of melamine exposure was longer in children with nephrolithiasis (high exposure group) than in controls (median duration 12 months versus 6 months). High melamine exposure was significantly associated with nephrolithiasis (odds ratio (OR) 61.04; 95 % CI: 12.73 – 292.84), and the risk increased with estimated melamine exposure level (p. for trend <0.001) (Wang et al., 2009). Significant factors associated with stones were urinary frequency, past history of urinary tract infection, family history of urolithiasis and having resided in China.

A cross-sectional study from Hong Kong investigated renal outcomes of children < 12 years (n=3170; 1422 girls, 1748 boys) after exposure for one month or more to milk products tainted with melamine. The authors noted that the resulting exposure to melamine was lower (0.01-0.21 mg/kg b.w. per day) than the FDA-derived TDI of 0.63 mg/kg b.w. per day. One child had a confirmed renal stone, seven were suspected of having melamine related renal deposits, and 6.6 % were positive for blood in urine by reagent strip testing. They were investigated further by ultrasonography, renal function, and urine microscopy. Only 7.4 % of those with blood in urine by reagent strip testing were confirmed by microscopy, suggesting an overall estimated prevalence of less than 1 % for microscopic haematuria. The estimated melamine intake of the eight children with renal stones or deposits was between 0.01 mg/kg per day and 0.21 mg/kg per day. The authors concluded that no severe adverse renal outcomes, such as acute renal failure or urinary tract obstruction, were detected in the children (Lam et al., 2009).

In a study from Shenzen Nanshan Hospital, China, 1091 children younger than 4 years who had been exposed to melamine contaminated milk were screened for nephrolithiasis. Urinary stones were detected by type B- ultrasonography, and renal status was examined by a routine urine test panel and a renal function test. Twelve children (10 boys, 2 girls) were diagnosed with kidney stones. Eleven of these 12 patients had consumed milk with a high level of melamine (955-2563 mg/kg, consumed for 1-12 months), 1 patient had consumed milk with lower melamine content (6.2-17.0 mg/kg, for 12 months) (Zhu et al., 2009).

In a study from Linyi, Shandong Province, China, the estimated melamine intake in 49 patients with nephrolithiasis and/or hydronephrosis, who were diagnosed in September 2008 and reinvestigated in December 2008, was reported. During the screening in September 2008, 63 cases of nephrolithiasis and/or hydronephrosis were identified among 3976 infants screened who had ingested melamine-tainted formula milk, and one case was identified among the 358 infants screened who had not ingested melamine tainted formula. It was assumed that the hydronephrosis was due to the nephrolithiasis, but this was not confirmed by other means. None of the patients had common risk factors for nephrolithiasis. Haematuria was found in 1 of 19 patients investigated who had not recovered, and in 2 of 30 who had recovered. The daily melamine intake in the 49 patients was estimated by multiplying formula intake by the highest reported melamine levels in each of the products, as reported by China's General Administration of Quality, Supervision, Inspection and The Quarantine (AQSIQ). For seven cases samples of different formula that had been consumed were provided. The melamine concentrations in the samples were far lower than those reported from AQSIQ, suggesting that the exposure estimations may have been on the high side. 14 of the patients had exposure to melamine only from formula feeding, and the rest had been exposed to melamine contaminated food in addition. Only exposure from formula intake was estimated. The daily melamine intake among all 49 patients ranged from 0.01 – 62.67 mg/kg body weight per day (median 0.90, geometric mean 1.28) and the intake among the 14 solely formula-fed ranged from 0.04 to 62.67 mg/kg body weight per day (median 0.61, geometric mean 0.92). Four of these 14 patients with stones had intake in the range 0.04 – 0.16 mg/kg body weight per day (Chen, 2009b), which is below the TDI of 0.2 mg/kg body weight.

At the Children's Hospital of Zhejiang, China, 562 of 15577 melamine-exposed infants and children screened by ultrasonography in September and October 2008 had urinary tract calculi. 846 subjects with detailed data were enrolled for further analysis and divided into calculus group (n= 326) and non-calculus group (n= 520), with age from one month to five years (median, 18 months). Long duration of formula feeding, consumption of formula with high melamine concentrations, and low water intake

were risk factors for calculi (p>0.05). Urinalysis was performed in 147 of the 326 children with calculi. Increased white blood cell count was noted in 18 children, occult blood was positive in 25, protein positive in 8, and increased blood creatinine was seen in 3 children (Zhang et al., 2009).

Nephrolithiasis in 683 cases with definitive imaging of stones in ultrasonography in different hospitals in Beijing between 14 and 24 September 2008 were revisited 25-30 September 2008. This group was compared with 6498 children without nephrolithiasis as diagnosed by ultrasonography, which were participating in the survey on childr'n's health and feeding status in Beijing, conducted between 25 September and 5 October 2008. All children were age <3 years. Data on current and past formula feeding as well as on other possible risk factors were also collected. The questionnaires, which were filled in by face-to-face interview, contained information of sex, age, birth (preterm or term), birth weight, urinary malformation, parents with a history of urinary stones, breast-feeding time, history of formula feeding, formula brand, time feeding started, cumulative time, quantity per months, and the total quantity.

The children were exposed to four of the 22 melamine contaminated brands that were identified by the General Administration of Quality Supervision, Inspection, and Quarantine of the People's Republic of China and, according to their report, the melamine contents in the four brands were 2563 mg/kg, 150 mg/kg, 53.4 mg/kg, and 12 mg/kg. Average individual daily melamine intake (g per day) from formula since birth was calculated and divided by kilo b.w. at birth and at investigation. The cumulative time of exposure and daily intake of melamine was higher amongst children with stones than amongst children without stones. Estimated dietary exposure divided by b.w at examination is summarised in Table 39. The OR of nephrolithiasis increased with daily melamine intake per kg body weight after adjustment for age, sex, preterm birth or not, low birth weight, urinary malformation, parents with a history of urinary stones, and cumulative time of exposure. The adjusted OR in the highest of the 10 exposed groups (based on current body weight) was 11.3 (95 % CI 5.9-21.8). The OR was increased also in the lowest of the 10 exposed groups (daily intake >0 to 0.2 mg/kg, adjusted OR 1.7, 95 % CI 1.3-2.4). The adjusted OR was generally lower, but still highly statistically significant when exposure was based on birth weight. If the exposure group >0 to 0.2 mg/kg was divided into the two groups >0-0.1 and >0.1-0.2 mg/kg, the adjusted OR in the two groups were 1.8 (95 % CI 1.3-2.5; P=0.001) and 1.7 (95 % CI 1.1-2.6; P=0.025), respectively, for daily intake based on current b.w. The authors concluded that their results showed an increasing risk of nephrolithiasis with increasing levels of daily melamine intake, starting at a level of intake lower than 0.2 mg/kg bw (Li et al., 2010).

Incidence of nephrolithiasis in infants related to average individual daily melamine intake from formula divided by both current weight and birth weight is shown in Table 40. The table also shows the mid-point in the exposure interval that were used by (Li et al., 2010) for calculation of OR.

Only exposure from milk formula has been calculated. Other food that the children eat (at least those more than 4-6 months old) and that might also contain melamine has not been taken into consideration. High melamine concentration in powdered milk products other than infant formula and in liquid milk and yogurt has been reported by the Chinese authorities (Gossner et al., 2009). There is thus reason to believe that the total dietary exposure has been higher since adulterated milk might have been used in the children's food. Of the children with stones, 66 % were 1-3 years old.

One could perhaps expect that older children would be in the lower exposure group because of lower milk formula consumption and higher body weight. If so, age would be a significant factor. This seems not to be the case, since increasing age does not alter the OR significantly. There is a lack of information on dietary habits of Chinese children, for example, whether it is common to use milk formula up to age 3 years. If the milk consumed is predominantly formula, the contribution from other food might be low in comparison.

**Table 39:** Summary of human studies on melamine intake and nephrolithiasis.

| Country, region, reference | Study design | Concentration in formula (mg/kg) or estimated daily dietary exposure (mg/kg b.w. per day) | Cases | Controls | Number screened | Age | Findings |
|---|---|---|---|---|---|---|---|
| China, Peking, Guan et al., 2009 | Cross-sectional | >500 mg/kg, <150 mg/kg, 0 | 50 confirmed, 112 suspected | | 589 | <26 mnt | Preterm birth, high melamine content in formula significantly associated with stones. |
| China, Guandong, Zhu et al., 2009 | Cross-sectional | 955-2563 mg/kg (11 patients), 6.2 – 17 mg/kg (1 patient) | 12 | | 1091 | <4 y | Stones associated with high melamine exposure |
| China, Shandong, Chen, 2009b | Cross-sectional | 0.01 to 62.67 mg/kg b.w. per day (all) 0.04-62.67 mg/kg b.w. per day (only formula fed) | 49 14 only formula fed | | 3976 | | Even low levels of melamine exposure may be a risk factor |
| China, Zhejiang, Zhang et al., 2009 | Case-control | Max concentration in 5 types of formula consumed: 2563, 150, 53, 17, and 12 mg/kg | 326 | 520 | 15577 | 1 mnt – 5 y | Significant risk factors for calculi: long duration formula feeding, high melamine content in formula, low water intake |
| Taiwan, Wang et al., 2009 | Case-control | >2.5 mg/kg, 0.05-2.5 mg/kg, <0.05 mg/kg | 14 | 28 matched controls | 1222 | <16 y, median in cases 2 y | High vs low exposure: OR for nephrolithiasis 61.04, 95 % CI 12.73-292.84. Significant factors associated with stones: urinary frequency, past history of urinary tract infection, family history of urolithiasis, having resided in China. |
| Hong Kong, Lam et al., 2008 | Cross-sectional | 0,01 to 0,21 mg/kg b.w. per day | 1 confirmed, 7 suspected | | 3170 | <12 y | No severe renal outcomes detected |
| Beijing, Li et al., 2010 | Case-control | 0 >0-0.2, >0.2-0.4; >0.4-0.8, >0.8-1.6, >1.6-3.2 , >3.2-6.4, >6.4-12.8, >12.8-25.6, >25.6-51.2 and>51.2 All doses expressed in mg/kg b.w. | 683 | 6498 | | <3 y | OR of nephrolithiasis increased with daily melamine intake after adjustment for age, sex, preterm birth or not, low birth weight, urinary malformation, parents with a history of urinary stones and cumulative time of exposure. OR was 11.3 for the highest exposure group The odds ratio was increased also in the lowest of the exposed groups (>0 to 2.2 mg/kg, adjusted OR 1.7). |

b.w.: body weight; mnt: months; y: years; CI: confidence interval: OR: odds ratio.

**Table 40:** Incidence of nephrolithiasis in infants and children and melamine intake from milk formula (based on data from Li et al., 2010) by estimated individual daily melamine intake (g per day) from formula since birth related to kilo b.w. at birth and at investigation.

| Incidence of nephrolithiasis related to birth weight | Incidence of nephrolithiasis related to b.w. at investigation [1] | Melamine intake mid-point (mg/kg b.w.per day) based on b.w. |
|---|---|---|
| 115/3062 | 115/3062 | 0 |
| 38/575 | 98/1334 | 0.1 |
| 41/475 | 59/542 | 0.3 |
| 37/456 | 76/590 | 0.6 |
| 67/567 | 58/340 | 1.2 |
| 70/539 | 37/235 | 2.4 |
| 51/288 | 25/182 | 4.8 |
| 36/200 | 54/305 | 9.6 |
| 22/202 | 81/342 | 19.2 |
| 72//346 | 64/202 | 38.4 |
| 84/416 | 16/47 | 76.8[2] |
| 50/138 | | 153.6[3] |

[1] Data used in dose response modelling in this opinion.
[2] Mid point of the highest interval for intake related to b.w. at investigation
[3] Mid point of the highest interval for intake related to b.w. at birth.

### 6.4.1. Estimates of intake of melamine among affected children

Individual data on infant formula consumption and melamine concentration in infant formula consumed by affected children are to a large extent not available.

A survey on occurrence of melamine and its analogues in infant formula samples from markets in the Gansu and Beijing provinces, including some samples from families with affected children in the Gansu province, which was heavily affected by the melamine incidence, found highest concentrations of melamine in Sanlu infant formula samples (mean 1673 mg/kg), with low concentration of cyanuric acid (mean 1.6 mg/kg), ammeline (1.7 mg/kg) and ammelide (2.9 mg/kg). Also raw material used for adulteration contained low mean concentration of cyanuric acid (7 mg/kg), ammeline (117 mg/kg) and ammelide (355 mg/kg) compared to melamine (178 571 mg/kg) (Wu et al., 2009b). These data were used to estimate dietary melamine exposure from tainted infant formula from mean body weight at age 3, 6, 12, and 24 months and maximal infant formula consumption, based on recommended usage in the package insert of different formula brands (Jia et al., 2009). Median concentration in all the 111 Sanlu milk samples from Beijing and Gansu analysed was 1000 mg/kg (range <0.05-4700 mg/kg) (Table 40). Median concentration in 52 Sanlu milk samples from the Gansu province was 1700 mg melamine/kg, 90$^{th}$ percentile was 2880 mg/kg and maximum was 4700 mg/kg. Estimated exposure based on recommended formula consumption and median contamination ranged from 9 to 23 mg/kg b.w. per day in the age groups 3-24 months, with highest intake at 3 months (Table 41). The Sanlu infant formula samples are not necessarily representative for all Sanlu milk and not all children with urolithiasis consumed Sanlu milk, other brands were also affected (Guan et al., 2009). The concentrations in the formula samples reported by Wu et al. (2009b) are however in the same range as those presented by Guan et al. (2009) Overall, the concentration in milk formula and calculated dietary intake are in line with the studies from China (Table 38).

**Table 41:** Melamine intake estimates (mg/kg body weight per day), based on Jia et al. (2009).

| Age | Body weight | Maximal consumption | Concentration in 111 Sanlu milk samples | | | |
|---|---|---|---|---|---|---|
| months | kg | Kg per day | Mean 1212 mg/kg | Median 1000 mg/kg | P90 2600 mg/kg | Maximum 4700 mg/kg |
| 3 | 5.5 | 0.13 | 29 | 23 | 61 | 110 |
| 6 | 7 | 0.15 | 26 | 21 | 56 | 101 |
| 12 | 10 | 0.15 | 18 | 15 | 39 | 71 |
| 24 | 14 | 0.12 | 10 | 9 | 22 | 40 |

P90: 90$^{th}$ percentile.

### 6.4.2. Evaluation of human data from the melamine incidence in China as basis for derivation of tolerable intake

The studies included in Table 39 cannot provide a reliable quantitative estimate of the incidence of urolithiasis resulting from melamine exposure, since the population representativity of children brought to hospitals for screening is unknown. The studies from Taiwan and Hong Kong refer to cases possibly connected to lower melamine concentrations in milk formula. However, the background incidence of renal calculi among children in the region has not been taken into consideration. According to Langman (2009), there are no published prevalence or incidences for nephrolithiasis in children. A recent review on pediatric urolithiasis highlights an apparent increase in cases in anecdotal and single centre reports (Mandeville and Nelson, 2009).

Weaknesses in the exposure assessments, in particular the lack of individual consumption data and actual melamine concentration in milk formula consumed and contribution from other food items makes it difficult to derive a benchmark dose (BMD) from most of the epidemiological data published after the melamine incidence. The exposure data are clearly better in the paper from Li et al. (2010) because of the individual weight records and knowledge of individual milk consumption. It is also a strength that only cases with stones confirmed by ultrasonography have been included in the study. However, the study only provides exposure from formula feeding. There are reasons to assume that the total dietary melamine exposure may have been higher in the children eating other food than milk formula because of possible adulteration of other foods on the market in China, e.g. cakes and biscuits. In addition there are questions connected to how representative the analyzed formula samples are, and this would increase the uncertainty connected to the exposure assessments. Concentrations in formula were varying, not only between brands but also within the same brand (Jia et al., 2009; Guan et al., 2009; Chen, 2009b). Furthermore, there is a possibility that parents that brought their children to hospital for examination over-reported the use and/or consumption of highly melamine-contaminated formula brands. The description of the control group in the Li et al. study (2010) was incomplete. Bearing these limitations in mind the CONTAM Panel has chosen to model the data in the study from Li et al. (2010) in order to explore the usefulness of the study to derive a BMD.

### 6.4.3. Urinary tract stones in children

The most common factors that induce childhood urinary calculi are metabolic abnormalities, urinary tract infection and urinary system deformities. In children the most common urinary calculi are those containing calcium; stones composed of calcium oxalate account for 45 % to 65 %, and those composed of calcium phosphate 14 % to 30 %. Uric acid, struvite and cystine stones account for 5 % to 10 % (Cameron et al., 2005).

#### 6.4.3.1. Urinary tract stones in children after the melamine incidence in China

Unlike renal stones with other causes in adults and children, several of the reports after the melamine incidence describe the absence of conventional symptoms and signs related to nephrolithiasis (hematuria, leukocyteuria, and other urinary abnormalities) in a large proportion of the children with

stones (Langman, 2009). The reason for the absence of symptoms remains to be explained. Except for larger stones, the melamine-containing deposits are generally not revealed by radiographic examination, and ultrasound examination has been used in order to detect kidney stones in children who have consumed melamine contaminated milk powder (Jia et al., 2009; Zhu et al., 2009; Lam et al., 2009). The melamine calculi are reported to be different from radiopaque calcium oxalate or phosphate calculi when examined by ultrasonography, by being less echogenic, having a more "sandy" appearance and being less dense. He et al (2009) reported that the posterior portion of the calculi could be observed and was accompanied with a feeble or absent acoustic shadow (He et al., 2009). In agreement with this Sun et al. (2009a,b) found two appearances by ultrasound examination: sand-like crystals, solitary or in clusters with no shadow or comet-like shadow, and larger sized, clump-like calculi with light shadow ( Sun et al., 2009a, b).

Jia et al. (2009) described the features of the calculi expelled from some patients to be brown or brown-yellow fine gravels, and some were lump-like in appearance. The larger ones were up to 1.0 cm × 0.5 cm and oval in shape. The surface of stones has been described as covered by a brown soft shell and with white inner part, and the shape of the stone could be changed on compression.

#### 6.4.3.2. Localization of melamine-associated deposits in the urinary tract

The deposits were mainly located in the renal pelvis and calyces, and at the pyelouretic junction, the site where the ureter spans the iliac blood vessels and at the ureter-bladder junction (Sun et al., 2009a, b). Ding (2009) found that most melamine associated stones were irregular and nubby in shape and were mainly localized to the renal pelvis.

#### 6.4.3.3. Characterisation of urinary stone composition in humans after the melamine incidence

In a case report, a urinary calculus from an 11 months old Chinese girl who had ingested melamine contaminated milk was studied by Fourier transform infrared spectrometry and scanning electron microscopy, coupled with x-ray microanalysis. Crystals composed of melamine, cyanuric acid and uric acid was prepared in vitro for comparison. Only the crystals composed of melamine and uric acid had features identical to those obtained from the calculus, indicating that cyanuric acid was not present in the calculus (Grases et al., 2009).

Chemical analysis (LC-MS) of the calculi expelled from 12 cases in a study from Beijing Children's Hospital, China showed that the main contents of the calculi were uric acid and melamine, with molar ratio 2:1 (Jia et al., 2009).

During the melamine incident in China in 2008, bladder and kidney precipitates observed in children were further examined. The precipitates expelled from 7 children were analysed by HPLC-MS and found to be composed of uric acid and melamine in a ratio of 1.2:1 to 2.1:1. No cyanuric acid was found (Sun et al., 2009a, b).

### 6.4.4. Cyanuric acid

No human data related to exposure to cyanuric acid has been identified, except from an early study on absorption and excretion in long-distance swimmers (Allen et al., 1982).

## 6.5. Dose-response modelling

Data suitable for dose response-modelling were only available for melamine, not for cyanuric acid, ammelide and ammeline.

From the hazard characterisation based on animal data (6.2) and human data (6.4), the Panel selected the following studies for the dose-response modelling of melamine:

- the two NTP studies on the incidence of urinary bladder stones in male F344 Fischer rats fed with melamine containing diet for 13 weeks (see Table 37).
- the data on the incidence of nephrolithiasis in infants related to average individual daily melamine intake from formula based on current birth weight (see Table 39).

The Panel noted that WHO (2009a) based its risk characterisation on the two NTP 13-week oral studies. WHO combined the two studies because of "*consistent responses for both bladder stone formation and the reported incidence of bladder epithelium hyperplasia*". Applying three dichotomous models only (quantal linear, multistage and Weibull), because of "*some underlying biological support for these three models*", WHO (2009a) reported a BMD of 531 and a BMDL of 415 mg/kg diet and converted to a BMDL of 35 mg/kg b.w. per day applying the standard dietary conversion factor of 0.10 for young rats (i.e. assuming 10 g food consumed per day) taking into account an "*additional feed reduction adjustment factor of 14 % as observed in the second subchronic study*". In contrast, the Panel calculated the mean intake during the studies in units of mg/kg b.w. per day directly from the concentration of melamine in feed, the weekly feed consumption and the weekly recorded body weights. In addition, the Panel used all quantal (dichotomous) dose response models available in the US Environmental Protection Agency (EPA) BMDS software, identified a set of acceptable models and concluded from there a reference point (see Appendix III and IV).

### 6.5.1. Dose-response data in animals

The NTP studies (NTP, 1983) are described in detail in section 6.2.1. In NTP study I, groups of 12 F344 rats were fed melamine at dietary concentrations of 0, 6000, 9000, 12 000, 15 000 and 18 000 mg/kg. Stones were found in the urinary bladders of males in all melamine-treated groups in a dose related manner (incidence: 0/12, 6/12, 8/12, 12/12, 10/12 and 12/12, respectively). Lower doses of melamine were administered in NTP Study II, with dietary concentrations of 0, 750, 1500, 3000, 6000 and 12 000 mg/kg, and 10 animals per group. All groups, including the control group, manifested bladder stones (incidence: 1/10, 2/10, 5/10, 7/10, 9/10 and 9/9, respectively. The two studies had the doses 6000 and 12000 mg/kg in common. NTP study I shows an incidence of 6/12 at 6000 mg/kg diet compared to 9/10 in NTP Study II; at 12 000 mg/kg diet both studies exhibited 100 % incidence (Table 37).

The Panel explored various ways of analysing the dose-response data from the two NTP studies using the BMD approach (Appendix III). For the modelling, the incidence of urinary bladder stones, as evaluated by NTP (1983), was the critical endpoint. The BMR was chosen as 10 %, the default response level for quantal response data as recommended in EFSA (2009) on the use of the BMD approach. The outcomes of that dose-response analysis are shown in Appendix IV-Tables A.1.1-A.1.4.

All quantal dose response models in the US EPA's benchmark dose software BMDS 2.1.1[37] were used for modelling the incidence of urinary bladder stones/crystals in the rats (see Appendix III and IV). Acceptability of a model was assessed using the log-likelihood value associated with the fitted model (when tested versus the full model). In general and in accordance with the Scientific Opinion of the EFSA (EFSA, 2009) a goodness-of-fit was judged as sufficient if the fit showed a probability value (p-value) not smaller than 0.1 (i.e. $p \geq 0.1$), using the log-likelihood ratio test . Models fulfilling the criterion were identified as accepted models.

The Panel concluded that the results of the second NTP 13 week study, with melamine at concentrations from 750 to 12000 mg/kg diet provide the most appropriate basis for deriving a reference point, based on the different dose response analyses. This is supported by the fact that the second study was performed by the NTP to find a no-effect level for urinary bladder stone formation

[37] http://www.epa.gov/ncea/bmds/about.html

(see page 19 of the NTP report.) which therefore better characterises the dose response relationship in the lower dose region which is important for deriving a $BMD_{10}$. All models gave an acceptable fit with ranges of $BMD_{10}$ values of 27.8-66.3 mg/kg b.w per day. The range of the respective benchmark dose lower confidence limit (BMDL) values was 18.8-47.1 mg/kg b.w per day after excluding two models (Weibull and Gamma) because of concerns on the validity of the fit, see Appendix IV).

Overall, the Panel concluded that the lowest $BMDL_{10}$ of 19 mg/kg b.w. per day was the appropriate reference point.

### 6.5.2. Dose-response data in humans

Almost all human studies reported so far in the available literature did not allow a dose-response analysis since exposure estimation appeared to be highly imprecise and affected by substantial bias, e.g. selection bias when the population has been chosen or reporting bias when the time and the amount of use of brand specific infant formulas were recorded and translated to individual exposure. Only the study by Li et al. (2010) provided dose-response data, characterising the prevalence of nephrolithias in children not older than 3 years in Beijing after the incident of melamine-contaminated formula in mid-2008, that appeared suitable for modelling (see section 6.4). Average daily intake per kg b.w. related to individual body weight at the time when children were examined for nephrolithiathis was used as the exposure metric for the BMD analysis. There were a total of 11 concentration intervals. The concentration midpoints of the geometrically increasing sequence of exposure intervals and the incidence of melamine induced urolithiasis quantified as the presence of urinary bladder stones/crystals in the children was used as reported in Table 39. The BMD analysis was performed for these prevalence data in the same way and according to the same criteria as described for the animal dose-response incidence data in 6.5.1 above. The BMR was chosen as 10 % which was at the low end of the observed dose response data. Four models gave an accepted fit with $BMD_{10}$ and $BMDL_{10}$ values of 1.10 - 1.28 and 0.74 - 0.84 mg/kg b.w. per day, respectively. The Panel concluded that the lowest $BMDL_{10}$ of 0.74 mg/kg b.w. per day was a reference point for the human data based on the prevalence of nephrolithiasis. The Panel noted that this BMD analysis was based on 7181 children sampled in a two step approach from a target sample of at least 41 000 children in the area of Beijing with a target population of 300 000 children. The outcome of the dose response modelling is dependent on the way in which the reference population for the 683 children with nephrolithiasis was defined. If a larger reference population was selected the $BMDL_{10}$ would be much higher than 0.74 mg/kg b.w. per day, and this value could be of the order of magnitude of a $BMDL_{01}$. Therefore, the $BMDL_{10}$ of 0.74 mg/kg b.w. per day was viewed only as a possible lower bound and not used for risk characterisation.

## 6.6. Derivation of tolerable daily intake(s) for melamine and cyanurate

### 6.6.1. Melamine

In line with the previous evaluations, the CONTAM Panel agreed that melamine is not genotoxic and therefore it is appropriate to establish a TDI. Formation of calculi or stones in the kidney was considered to be the relevant critical effect observed at lowest doses. These can result in proximal tubule damage which could have longer term health impacts. The data related to illness in humans due to adulteration of infant formula was not the preferred basis for deriving a TDI due to the uncertainties in the exposure assessments, including possible over-reporting of formula consumption, differences in melamine concentration in the formula and absence of information on exposure from foods other than infant formula. The $BMDL_{10}$ calculated by the CONTAM Panel was 19 mg/kg b.w. per day based on the low dose NTP study in rats, and this was used as the starting point in establishing a TDI for melamine.

The CONTAM Panel considered whether the default uncertainty factors of 10 to allow for inter-species differences and 10 for intra-species differences would be adequate. In principle additional

factors should be considered if there are important gaps in the database, or if there is evidence that the individual 10-fold factors are insufficient to allow for inter- and intra-species differences. The $BMDL_{10}$ was derived from the 13-week low dose feeding studies in rats. A 2-year feeding study in rats and re-examination of that study (NTP, 1983; Melnick et al., 1984; Hard et al., 2009) demonstrated that transitional cell carcinomas of the urinary bladder were associated with the presence of bladder stones, supporting a non-genotoxic mechanism of carcinogenicity. The study showed a significant increase in the incidence of reflux nephropathy (7/50 vs 1/49 in control) but not of bladder stones (1/50 vs 0/45 in controls) at 126 mg/kg b.w. per day melamine, which is about 8 times higher than the $BMDL_{10}$. Taking into account the steep dose response relationship, this comparison suggests that there is no need for an additional uncertainty factor to allow for extrapolation from 13-week to chronic studies.

However, there are a number of reasons for considering possible increased susceptibility of humans, particularly infants, compared to rats. Melamine can form crystals with uric acid in a number of species including rats and humans (see section 6.1.5.2). Humans lack the enzyme urate oxidase, which metabolises uric acid to allantoin. The uric acid concentration of human urine is typically 2-3-fold that of rats (see Table 35) and the uric acid excretion adjusted to the glomerular filtration rate of neonates at term is about 4-fold greater than that of older children or adults (see Table 36). The uric acid excretion in pre-term infants appears to be greater and more variable. The pH of human urine has been reported to be typically in the region of 5.0-6.0, although lower and higher values may occur under some circumstances. This compares to 7.3-8.5 reported in rats. As discussed in section 6.1.5.2, formation of melamine-urate crystals is most likely to occur at acid pH since the pKa values of melamine and uric acid are 5.5 and 5.8 respectively. The CONTAM Panel estimated (section 6.1.5.2) that 7.8 % of melamine would be complexed with uric acid in rat urine and 80 % would be complexed in the urine of infants, there is therefore an approximately 10-fold higher propensity for the melamine-uric acid complex to form in infants than in rats, as a result of the lower pH and the higher excretion of uric acid. This difference would be smaller if the pH of rat urine was lower.

The CONTAM Panel considered the appropriateness of the default 100-fold uncertainty factor (inter-species and intraspecies uncertainty factors) for melamine, particularly taking into account the lack of metabolism, and the impact of urinary uric acid and pH on the formation of the melamine complexes with uric acid. Based on the information summarised in the paragraphs above, a factor of 10 is adequate for differences in melamine-uric acid complex formation in infants compared with rats, as a result of the lower pH and the higher excretion of uric acid. Melamine is fully absorbed and eliminated essentially un-metabolised in the urine of both rats and humans. Therefore, there is no additional toxicologically relevant interspecies variability

The inter-species uncertainty factor is based on differences between rats and human infants (including full term neonates), the most sensitive relevant human subgroup, in melamine-uric acid complex formation, which might suggest that an intra-species uncertainty factor is not required. However, it is also necessary to take into account the increased susceptibility of the infant kidney (size of the kidney, tubular diameter as smaller tubules have a greater probability of being damaged by crystals than the larger ones of the adult kidney). The default value of 10, normally assigned for the intra-species uncertainty factor for toxicokinetic and toxicodynamic variability, was considered to be adequate.

Overall, the CONTAM Panel concluded that an uncertainty factor of 100 was adequate to account for intra- and inter species differences (which includes infants) when deriving a TDI for melamine. Dividing the $BMDL_{10}$ of 19 mg/kg b.w. per day by this uncertainty factor, with rounding to a single significant figure results in a TDI of 0.2 mg/kg b.w. for melamine. Due to higher urinary levels of uric acid in premature infants, their potential greater sensitivity to melamine arising from greater immaturity of kidney function and other unquantifiable susceptibilities, this TDI does not apply to premature infants.

Since the WHO expert meeting in December 2008, additional data had been published regarding the exposure of infants to melamine in the Chinese milk formula incident. Whilst there are considerable uncertainties with respect to the exposure estimates in most of these reports, a $BMDL_{10}$ of 0.74 mg/kg b.w. per day can be calculated from the data of Li et al. (2010), as described in section 6.5. This is nearly 4-fold higher than the TDI. Taking into account that the effects of melamine are due to the concentration in urine and the steepness of the dose-response relationship, this human $BMDL_{10}$ provides supporting evidence for the adequacy of the TDI to protect infants.

### 6.6.2. Melamine analogues

A TDI for cyanuric acid of 1.5 mg/kg b.w. was proposed in a provisional EFSA statement (EFSA, 2007), based on the JECFA evaluation of the disinfectant dichloroisocyanurate (WHO, 2004). The TDI was derived from the results of a 2-year study in which sodium cyanurate was administered in drinking water to rats. Lesions of the urinary tract (including calculi and renal tubular nephrosis) as well as secondary uremia-related heart lesions were reported in the males of the highest dose group (5375 mg/L sodium cyanurate in drinking water, equivalent to 371 mg/kg b.w. per day). The NOAEL was 2400 mg/L, equivalent to 154 mg/kg b.w. per day sodium cyanurate. An uncertainty factor of 100 for inter- and intra- species differences was applied to the NOAEL to establish the TDI of 1.5 mg/kg b.w. The CONTAM Panel did not identify newer studies to indicate that this TDI was no longer appropriate. However, it was noted that this TDI is based on sodium cyanurate, and is equivalent to 1.3 mg/kg b.w. cyanuric acid. This is a more accurate value for the TDI, but is not toxicologically different from 1.5 mg/kg b.w.

The toxicological databases for ammelide and ammeline are extremely limited. However, as noted by WHO (2009a), it is expected that each alone would not be more toxic than melamine.

### 6.6.3. Combination of melamine with analogues

Based on the observations in animals that received contaminated feed, and on the results of more recent experimental investigations, it is clear that co-exposure to melamine and cyanuric acid is more toxic than exposure to melamine alone. Whilst they have been subject to fewer studies, there is limited evidence that ammelide and ammeline can also co-precipitate with melamine.

As discussed in section 6.2.4, the available studies allowing direct comparison indicate that a combination of melamine and cyanuric acid produced similar toxicity to a 12-20 fold higher dose of melamine administered alone. However, it is unclear whether a factor of 20 would also result for different dose combinations of mixtures of melamine and its analogues, or for longer durations of exposure.

Thus the TDI for melamine is not appropriate if there is significant concomitant exposure to cyanuric acid, ammelide or ammeline. Such concomitant exposure could result in considerable health impact and the currently available data are inadequate to establish a TDI for this scenario.

## 7. Risk characterisation

### 7.1. Animal health risk assessment

Reliable no-observed-adverse-effect-levels (NOAELs) or lowest-observed-adverse-effect-levels (LOAELs) for melamine and cyanuric acid are not available for most species.

Exposure to melamine has been estimated for livestock based on the European action level of 2.5 mg/kg with a lower level of 0.5 mg/kg and a higher level of 10 mg/kg in mixed feed and in premixes. Of the ruminants, dairy goats and dairy cows would be the most exposed to melamine (42 and 40 µg/kg b.w. per day, respectively if compound feed contained 2.5 mg/kg melamine, or 168 and

160 µg/kg b.w. per day, if compound feed contained 10 mg/kg melamine respectively). Dose response data on tolerance in cows are not available, however one experimental report states that 27 mg/kg b.w. per day does not cause adverse effects which is approximately 170-fold above the exposure that would result from 10 mg/kg melamine in compound feed. In addition, cows and other herbivory species have an alkaline urinary pH, and even under the assumption of co-exposure to melamine and cyanuric acid, crystal formation is unlikely to occur.

In pigs, no adverse effects were observed after three days of exposure to melamine at a target dose of 400 mg/kg b.w. per day. The NOAEL is 3 to 4 orders of magnitude higher than the exposure of finishing pigs to 370 and 93 µg/kg b.w. per day melamine predicted to result from a concentration of 10 or 2.5 mg/kg melamine in feed respectively.

In tilapia fish, 3 days exposure to melamine did not result in adverse effects at doses ranging from 300-456 mg/ kg b.w per day. This NOAEL is 3 to 4 orders of magnitude higher than the exposure of 0.2 and 0.05 mg/kg b.w. per day predicted to result from a concentration of 10 or 2.5 mg/kg melamine in feed respectively.

Limited data indicate that a NOAEL for melamine alone in cats could be in the region of 180 mg/kg b.w. per day. This is 1500-fold higher than the predicted exposure of 120 µg/kg b.w. per day for canned food containing 2.5 mg/kg melamine, and 375-fold higher than the predicted exposure of 480 mg/kg b.w per day for canned food containing 10 mg/kg melamine. Due to the physiological characteristics of their kidneys and the high protein (needs and) content of their diet cats (*felidae*) have to be considered as the most sensitive animal species, followed by dogs (*canidae*). As yet all data from clinical cases report co-exposure to both melamine and cyanuric acid (scrap melamine) but, despite the numerous clinical reports, no conclusive exposure assessment was available and data from previous surveys are conflicting. However, an overall exposure level of 10 mg/kg melamine alone in canned or pet food is not expected to pose a risk to pets.

Exposure to cyanurate in ruminants has been estimated from a urea-based feed additive product defining levels of cyanurate up to 200 mg/kg and assuming the use of 30 g per 100 kg animal, giving a daily intake of 60 µg/kg b.w per day of cyanurate. A NOAEL for cynaurate of 600 mg/kg b.w. per day has been identified from the literature available in sheep and is approximately 10 000-fold above the exposure that would result from the use of such a feed additive and therefore presenting no risk to animal health.

Overall, estimated exposures to melamine or cyanuric acid individually at the scenarios of 0.5, 2.5 (the EU action level for melamine) and 10 mg/kg in feed are not expected to pose a risk to livestock, fish or pets (cats and dogs).

### 7.2. Human health risk assessment

The time scale for development of renal damage in humans resulting from melamine co-precipitates in urine is unclear but it is expected that it could arise as soon as precipitates are formed in the kidneys. This expectation is supported by observations in cats. Nephrotoxicity related to crystal precipitation is generally progressive, involving inflammatory reactions following the damage of the tubular epithelium, local ischemia and oxidative stress leading to irreversible tubular necrosis. The CONTAM Panel compared the TDIs for melamine and for cyanurate with the estimated dietary exposures that could result from both the 95$^{th}$ percentile as well as the mean of upper bound occurrence of melamine and cyanurate in food, as reported by the food industry.

For adult high consumers, the dietary exposure estimates to melamine in different EU countries based on the upper bound occurrence values are all below 11 µg/kg b.w. per day. For infants fed solely on formula, the dietary exposure estimates to melamine are all below 2 µg/kg b.w. per day. Melamine from metabolism of cyromazine has been estimated from residue studies in sheep at the levels of the MRL for cyromazine (300 µg/kg of meat) and would reach a maximum of 4 µg/kg in the meat. This

would result in an exposure to melamine of less than 0.02 µg/kg b.w. per day considering meat consumption of 300 g in a 60 kg adult. Contamination of the feed of laying hens with 10 mg/kg of melamine and a maximum transfer rate into eggs of 3.2 % would lead to residues in eggs resulting in an exposure in adults below 1 µg/kg b.w. per day after consumption of 100g of eggs per day. Overall, these estimated exposures are all well below the TDI for melamine of 0.2 mg/kg b.w. per day, and do not raise concerns for the health of consumers.

For adult high consumers, the dietary exposure estimates to cyanurate in different EU countries based on the upper bound occurrence values are below 16 µg/kg b.w. per day. For infants fed solely on formula, the estimated dietary exposures to cyanurate are all below 6 µg/kg b.w. per day. These dietary exposure estimates are all well below the TDI for cyanurate of 1.3 mg/kg b.w., and do not raise concerns for the health of consumers.

Melamine exposure resulting from the migration of food contact materials was estimated according to two scenarios (A and B), using the database on individual food consumption data for children" (EXPOCHI) for 13 different Member States and typical and high migration levels of melamine from a "standard" melaware article under typical (Scenario A) and severe time and temperature conditions (Scenario B), respectively. Exposure was calculated assuming that any food or beverage could come into contact with high melamine-releasing melaware articles and obtained by summing up, within each day, the exposure from all food groups for Scenario A. For scenario B only exposure from the one food item giving the highest exposure estimate within one day was considered. In this Scenario exposure to melamine from other food items was considered not to occur within the same day. As limited migration data are available for melamine issued from melaware and can coatings, the CEF Panel used conservative assumptions on migration to estimate exposure via food.

Total exposure to melamine in one day considering typical migration levels (scenario A) ranged from 30 to 80 µg/kg b.w. per day (mean) and from 50 to 120 µg/kg b.w. per day ($95^{th}$ percentile). Exposure in one day from one food/beverage considering high migration levels (scenario B) ranged from 40 to 110 µg/kg b.w. per day (mean), and from 70 µg to 230 µg/kg b.w. per day ($95^{th}$ percentile). These $95^{th}$ percentile estimates are below or slightly above the TDI. In view of the conservative assumptions, the CEF Panel considers that the few exposure estimates (210- 240 µg/kg b.w. per day) above the TDI do not raise a safety concern, although it recognises that excursion above the TDI is by definition undesirable.

The above conclusions only apply if there is reasonable confidence that exposure is essentially to melamine or cyanuric acid alone. If there is a possibility of significant co-exposure to melamine, cyanurate, ammelide or ammeline health impact could arise at intakes of melamine well below the TDI.

## 8. Uncertainty analysis

The evaluation of the inherent uncertainties in the assessment of exposure to melamine and cyanuric acid has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006b). In addition, the report on "Characterizing and Communicating Uncertainty in Exposure Assessment" has been considered (WHO/IPCS, 2008). According to the guidance provided by the EFSA opinion (2006b), the following sources of uncertainties have been considered: assessment objectives, exposure scenario, exposure model, and model input (parameters).

### 8.1. Assessment objectives

The objectives of the assessment were clearly specified in the terms of reference. The CONTAM Panel assessed the occurrence data that were collected by EFSA, and re-assessed the TDI on melamine and cyanuric acid in view of the outcome of the WHO Expert meeting. The co-exposure of animals and humans to melamine and structurally related compounds such as cyanuric acid, ammelide and

ammeline was considered. For this aspect, there is considerable uncertainty because of the limited toxicological database on co-exposure of melamine and structural analogues and no NOAEL can be identified or BMD model could be implemented.

### 8.2. Exposure scenario and exposure model

The occurrence data provided were to a large extent upper bound values, which leads to an overestimation of exposure. Moreover, it is uncertain whether the occurrence data are representative of the food commodities across the EU market. The uncertainty of the analytical methods was not reported, so the CONTAM Panel was not able to determine its influence on exposure calculation uncertainty. Further uncertainty in the exposure scenario for melamine and cyanuric acid in humans may be ascribed to the broad food categories considered in the EFSA concise database. The conservative approach in the exposure scenarios chosen by the CONTAM Panel could result in overestimation of exposure to melamine and cyanuric acid, but it is not possible to quantify this overestimation. There is also a lack of data on co-occurrence of melamine and structural analogues.

Uncertainty for the exposure assessment in pets (cats and dogs), livestock and fish is high since a number of assumptions have to be made regarding body weight, and food consumption patterns. Additionally, there is a lack of dose response data for livestock, fish and pets on melamine alone and co-exposure with its structural analogues.

### 8.3. Model input (parameters)

The data from experimental animals provide a suitable basis for the risk characterisation of melamine alone because of the relevance of the toxicological endpoint to the human situation (crystal formation in the kidney). The appropriateness of the models used for the interaction of melamine with an endogenous substance (uric acid) is a source of uncertainty. Inter- and intra-species differences due to urinary pH and uric acid content have been allowed for by assigning a 100-fold uncertainty factor. The TDI derived from animal data is supported by the available human dose response data. The human $BMDL_{10}$ is subject to high uncertainty due to the weaknesses of the exposure assessment (for details see section 6.4). There is uncertainty arising from the choice of the datasets and the dose-response models (including constraints of the model parameters) used for the BMD analysis. The modelling was conducted in line with the approach of the EFSA Scientific Committee (EFSA, 2009). The lowest $BMDL_{10}$ from the range of models with an acceptable fit was selected as the basis for the TDI, which is a conservative approach.

### 8.4. Summary of uncertainties

A summary of the uncertainty evaluation is presented in Table 42, highlighting the main sources of uncertainty and indicating an estimate of whether the respective source of uncertainty might have led to an over- or underestimation of the exposure or the resulting risk.

**Table 42:** Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of the dietary exposure to melamine.

| Sources of uncertainty | Direction[(a)] |
|---|---|
| Applicability of the analytical methods used for melamine and structural analogues | +/- |
| Representativity of the occurrence data for food across the EU market | +/- |
| Use of upper bound occurrence data in the exposure assessment | + |
| Assumptions on 'typical' and 'high' migration levels for food contact materials | + |
| Aggregation of food groups in the EFSA concise database for human exposure assessment | + |
| Assumptions on frequency of use of melamine-releasing food contact materials (principally melaware) | +/- |
| Assumptions for exposure assessment in animals regarding body weight, and food consumption patterns | +/- |
| Derivation of reference point based on animal studies and relevance of uncertainty factor of 100 for inter- and intra-species differences | + |
| Selection of lowest $BMDL_{10}$ from the range of models with an acceptable fit | + |
| Long term sequelae of the tubular damage due to crystal formation in infants | - |
| Appropriateness of the models used for the interaction of melamine with an endogenous substance,(uric acid) | +/- |
| Limited occurrence and toxicological data for co-exposure to melamine and structural analogues | - |

(a): + = uncertainty with potential to cause over-estimation of exposure/risk; - = uncertainty with potential to cause under-estimation of exposure/risk

The CONTAM Panel concluded that, despite the above uncertainties, by using the upper end of the high exposure estimate and the lower end of the $BMDL_{10}$ estimates, the risk assessments of melamine and of cyanuric acid individually are likely to be conservative.

## CONCLUSIONS

### *Uses and chemistry*

- Melamine is a high volume chemical that can be present in food as a result of approved uses in food contact materials, including melaware, can coatings, paper and board and adhesives. It may occur in food as a result of its use as a flame retardant. It is also a minor metabolite and degradation product of the pesticide and veterinary drug, cyromazine.
- Depending on the purification process, melamine may contain different levels of the structurally related substances cyanuric acid, ammeline and ammelide.
- Cyanuric acid residues can occur in food as a result of the use of dichloroisocyanurates as a source of active chlorine in disinfection agents.
- Melamine and cyanuric acid can be present as impurities in urea-based animal feed.
- Food and feed have been adulterated with high levels of melamine in order to inflate the apparent protein content.
- Due to its physicochemical properties, melamine can form complexes with its structural analogues and with other substances such as uric acid. Complex formation is highly pH dependent.
- The most sensitive and selective analytical method to measure melamine and its structural analogues is liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

***Occurrence and Exposure Assessment***

- EFSA received 2239 data on melamine in food and feed from European countries. These were the results of targeted sampling focussed on products where adulteration was considered likely. Because they were not representative of background levels, they were not considered appropriate for the assessment of background dietary exposure to melamine. No data on cyanuric acid were provided by European countries.

- The food industry provided 18546 and 502 data on melamine in food and feed, respectively, with 8061 and 308 data on cyanuric acid in food and feed, respectively. Most of the values reported appeared to be at the limit of detection or the limit of quantification. The data for food were used in estimating upper bound dietary exposure in humans after excluding a small number of samples with high values that were related to incidents of adulteration.

- Animal exposure to melamine was estimated for livestock based on the European action level of 2.5 mg/kg in mixed feed and in premixes, with lower and higher scenarios of 0.5 and 10 mg/kg, a range encompassing the data submitted by industry. Dairy cows and dairy goats would be the ruminants most exposed to melamine (40 and 42 µg/kg body weight (b.w.) per day and 160 and 170 µg/kg b.w. per day at 2.5 mg/kg and 10 mg/kg in compound feed respectively). Poultry (broilers and laying hens) would be the monogastric livestock most exposed to melamine (179 and 151 µg/kg b.w. per day and 714 and 605 µg/kg b.w per day respectively at 2.5 mg/kg and 10 mg/kg in compound feed).

- Cyanurate exposure was estimated in ruminants from a urea-based feed additive product defining levels of cyanurate up to 200 mg/kg and, assuming the use of 30 g as per 100 kg animal, giving a daily intake of 60 µg/kg b.w. per day of cyanurate.

- For adult high consumers, the dietary exposure estimates to melamine in different European Union (EU) countries based on the upper bound occurrence values are below 11 µg/kg b.w. per day. For infants fed solely on formula, the dietary exposure estimates to melamine are all below 2 µg/kg b.w. per day.

- For adult high consumers, the dietary exposure estimates to cyanurate in different EU countries based on the upper bound occurrence values are below 16 µg/kg b.w. per day. For infants fed solely on formula, the dietary exposure estimates to cyanurate are all below 6 µg/kg b.w. per day.

- Because of the very low values in the occurrence data and upper bound approach, the analysis of major food group contributors to the dietary exposure to melamine and cyanuric acid is mainly driven by the consumption data.

- Melamine residues in sheep resulting from cyromazine at the levels of the maximum residue level (300 µg/kg of meat) would reach a maximum of 4 µg/kg in meat. This would result in an exposure to melamine of 0.020 µg/kg b.w. per day considering meat consumption of 300 g in a 60 kg adult.

- Using the exposure scenario of 10 mg/kg of melamine in poultry feed, the maximum transfer rate (1.5-3.2 %) from experimental studies in laying hens and a high consumption of 100 g of eggs in adults, human dietary exposure from eggs would not be above 5 µg/kg b.w. per day.

- Melamine exposure resulting from the migration of food contact materials was estimated according to two scenarios (A and B), using the database on individual food consumption data for children" (EXPOCHI) for 13 different Member States and typical and high migration levels of melamine from a high-releasing melaware article under typical (Scenario A) and severe time and temperature conditions (Scenario B), respectively. Exposure was calculated

assuming that any food or beverage could come into contact with high melamine-releasing melaware articles and obtained by summing up, within each day, the exposure from all food groups for Scenario A. For scenario B only exposure from the one food item giving the highest exposure estimate within one day was considered. In Scenario B, exposure to melamine from other food items was considered not to occur within the same day. As limited migration data are available for melamine issued from melaware and can coatings, conservative assumptions on migration were used to estimate exposure via food. Total exposure to melamine in one day considering typical migration levels (scenario A) ranged from 30 to 80 µg/kg b.w per day (mean) and from 50 to 120 µg/kg b.w. per day (95$^{th}$ percentile). Exposure in one day from one food/beverage considering high migration levels (scenario B) ranged from 40 to 110 µg/kg b.w per day (mean) and from 70 µg to 230 µg/kg b.w. per day (95$^{th}$ percentile).

- Animal exposure to melamine was also estimated for pet animals with scenarios based on melamine concentrations of 0.5, 2.5 and 10 mg/kg, and consumption based on protein requirements in canned food (average: 48g/kg) or dried food (average: 13.5g/kg). Predicted exposure in cats and dogs is 120 µg/kg b.w. per day for canned food and 63 µg/kg b.w. per day for dried food containing 2.5 mg/kg melamine. At 10 mg/kg melamine, predicted exposure is 48 µg/kg b.w per day for canned food and 0.25 mg/kg b.w. per day for dried food.

***Toxicokinetics***

- Melamine is rapidly absorbed from the gastrointestinal (GI)-tract and rapidly excreted from the body with little or no metabolism and short half life (4-5 hours) in the rat and rhesus monkey. No significant accumulation of melamine in tissues is expected and levels in tissues are likely to be similar to plasma levels.

- Transfer of melamine from feed to pig muscle meat, liver and kidney is approximately 2 % (calculated as the ratio of concentration in tissues and in feed), but is very dependent on the time of sampling due to the rapid elimination resulting in lower transfer rates. Melamine is transferred to cow's milk with a worst-case estimate of 2 % of the daily dose. The highest estimated transfer factor for chicken meat and kidney would amount to 0.6 and up to 2.6 % respectively. Experimental studies with laying hens, which were given feed contaminated with melamine demonstrated transfer rates from feed to eggs varying between 1.5 and 3.2 %.

- The limited information available for cyanuric acid also indicates rapid absorption from the GI tract and rapid elimination via the urine with little or no biotransformation. Plasma half-lives of maximally a few hours have been reported.

- No data on kinetics of ammeline and ammelide have been found other than the observation that ammeline was tentatively identified as metabolite of cyromazine in chicken eggs.

- Exposure to melamine results in formation of crystals in the urinary tract, consisting of complexes of melamine with substances such as uric acid that occur naturally in urine, or with cyanuric acid if co-exposure occurs. Formation of these complexes is highly dependent on the concentration of melamine and on the composition of the urine (e.g. pH, uric acid, protein).

- Co-precipitation of melamine with uric acid is more likely to occur in humans because they excrete more uric acid in the urine than most mammals due to a lack of the enzyme urate oxidase. In neonates, the excretion of uric acid in the urine is higher than in adults. In addition, the urinary pH is lower in humans than that of rodents, increasing the likelihood of the formation of insoluble complexes between melamine and uric acid.

***Toxicity of melamine and structural analogues***

- The inherent toxicity of melamine is low. However, as a consequence of its physicochemical properties melamine forms crystals with other substances in urine. These crystals cause proximal tubular damage. Crystals have been observed in experimental animals, as well as in animals and human infants as a result of incidents involving adulteration of feed and infant formula with melamine, leading to fatalities in some instances.

- The Panel on contaminants in the food chain (CONTAM Panel) concluded that the data related to illness in humans due to adulteration of infant formula was not the preferred basis for deriving a tolerable daily intake (TDI) due to the uncertainties in the exposure assessments, including possible over-reporting of formula consumption, differences in melamine concentration in the formula and absence of information on exposure from foods other than infant formula.

- A 13-week study with dietary exposure of male rats to melamine provided the best basis for characterising the dose-response relationship in experimental animals. The Panel identified, for a 10 % increase in the incidence of urinary bladder crystals, a benchmark dose ($BMD_{10}$) of 41 mg/kg b.w. per day and its lower confidence limit ($BMDL_{10}$) of 19 mg/kg b.w. per day.

- The Panel considered the factors that could contribute to inter- and intra-species differences in the effects of melamine and concluded that an uncertainty factor of 100 was appropriate for deriving a tolerable daily intake (TDI).

- The CONTAM Panel considered that the TDI set by Scientific Committee for Food is no longer appropriate. A TDI of 0.2 mg/kg b.w. was derived by dividing the $BMDL_{10}$ of 19 mg/kg b.w. per day by the uncertainty factor of 100, with rounding to a single significant figure. This TDI is considered appropriate for infants, including neonates except for those born prematurely due to their higher urinary uric acid levels and greater immaturity of kidney function.

- From the human data, the Panel calculated a $BMD_{10}$ of 1.1 mg/kg b.w. per day and a $BMDL_{10}$ of 0.74 mg/kg b.w. per day for a 10% increased prevalence of nephrolithiasis. Taking into account that the effects of melamine are due to the concentration in urine and the steepness of the dose-response relationship, this human $BMDL_{10}$ provides supporting evidence for the adequacy of the TDI to protect infants.

- A TDI for cyanuric acid of 1.3 mg/kg b.w. was established based on a previous evaluation of the disinfectant dichloroisocyanurate.

- The toxicological databases for ammelide and ammeline are extremely limited and hence no TDI could be established.

- Co-exposure to melamine and cyanuric acid is more toxic than exposure to melamine alone due to the formation of crystals. There is limited evidence that ammelide and ammeline can also form crystals with melamine. The currently available information does not allow identification of a factor by which the toxicity is increased by co-exposure.

- The TDI for melamine is not applicable if there is significant concomitant exposure to cyanuric acid, ammelide or ammeline.

***Adverse effects in livestock, fish and pets***

- Limited data on adverse effects in livestock, fish and pets are available from the literature. In pigs, no adverse effects were observed after three days of exposure to melamine at a target dose of 400 mg/kg b.w. per day. In tilapia fish, 3 days exposure to melamine did not result in

adverse effects at doses ranging from 300-456 mg/ kg b.w. per day. Limited data from dietary short term exposure in cats (10 days) indicate that a NOAEL for melamine alone could be in the region of 180 mg/kg b.w. per day.

- In sheep, cyanuric acid exposure for 77 days resulted in no adverse effects at doses from 198 mg/kg b.w. to 600 mg/kg b.w. per day. In pigs, no adverse effects were observed after exposure to 400 mg/kg b.w. per day cyanuric acid for three days whereas in tilapia fish no adverse effect levels ranged from147-390 mg/ kg b.w. per day.

- Ammelide was not acutely toxic and did not show toxicity at 372 mg/kg b.w. per day when fed to sheep for a period of 6 weeks. An average daily intake of 296 mg ammeline/kg b.w. per day and 97 mg/kg b.w. of a mixture of ammeline and ammelide for 42 days respectively in the diet of sheep caused the death of half of the animals. No other data on ammelide and ammeline toxicity were available for livestock, pets and fish.

- Co-exposure to melamine and cyanuric acid in livestock, pets and fish showed higher toxicity compared with melamine or cyanuric acid alone with renal crystals formation in pigs, cats, catfish, trout and tilapia with lowest-observed-adverse-effect-levels values of 400, 32, 20, 7 respectively and NOAEL values in tilapia of 3-4 mg/kg b.w. per day.

***Animal health risk characterisation***

- The estimated exposures to melamine and cyanuric acid individually at the scenarios of 0.5, 2.5 and 10 mg/kg in feed are well below the doses causing toxicity and are therefore not expected to pose a risk to livestock and fish.

- Although quantitative data are limited, exposure to melamine or cyanuric acid individually at a level of 10 mg/kg in feed is not expected to pose a risk to pets (cats and dogs).

***Human health risk characterisation***

- Dietary exposure to melamine and cyanuric acid individually estimated from the available data relating to background sources is well below the respective TDIs and does not raise concerns for the health of consumers.

- This conclusion only applies if there is reasonable confidence that exposure is essentially to melamine or cyanuric acid alone. If there is a possibility of significant co-exposure to melamine, cyanurate, ammelide or ammeline, health impact could arise at intakes of melamine well below the TDI.

- When using conservative migration scenarios, dietary exposure of children to melamine from food contact materials such as melaware was estimated to be below or slightly above TDI for melamine. However, due to the conservative character of these estimates, a health concern was not identified.

**RECOMMENDATIONS**

- It is recommended that the current migration limit for melamine from food contact plastics is reconsidered in the light of the TDI of 0.2 mg/kg b.w. taking into account all sources of exposure.

- There is a need for co-occurrence data for melamine and its structural analogues (cyanuric acid, ammelide, ammeline) in food and feed.

- There is a need for additional information on the dose response relationships for combinations of melamine and its structural analogues (cyanuric acid, ammelide, ammeline).
- Development of a physiologically-based toxicokinetic toxicodynamic model is needed to improve the dose response modelling.

## REFERENCES

AFSSA (French Food Safety Agency), 2009. Summary of the Report of the 2006/2007 Individual and National Study on Food Consumption 2 (INCA 2). Available frosa.fr/Documents/PASER-Sy-INCA2.pdf.

Akrimajirachoote N, Poolperm P and Boonsoongnern A, 2008. Effect of feed contaminated with melamine on nursery pigs: a case report. Proceedings of the 46th Kasetsart University Annual Conference, Kasetsart, 29 January - 1 February, 2008. 650-657.

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

Altona R and MacKenzie H, 1964. Observation on cyanuric acid as a source of non-protein nitrogen for sheep. Journal of the South African Veterinary Medical Association, 35, 203.

Andersen W, Turnipseed S, Karbiwnyk C, Clark S, Madson M, Gieseker C, Miller R, Rummel N and Reimschuessel R, 2009. 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.

Bann B and Miller SA, 1958. Melamines and derivatives of melamine. Chemical Reviews, 58, 131-172.

Barbee SJ, Cascieri T, Hammond BG, Inoue H, Ishida H, Wheeler AG, Chadwick M, Hayes AW, McCauley J and McComish M, 1984. Metabolism and disposition of sodium cyanurate in the dog. Toxicologist, 4, 92.

Barbee SJ, Cascieri T, Hammond BG, Inoue T, Ishida N, Wheeler AG, Chadwick M, Hayes D, Macauley J and McComish A, 1983. Metabolism and disposition of sodium cyanurate. Toxicologist, 3, 80.

Battle D and Shah M, 2007. Physiological principles in the clinical evaluation of electrolyte, water and acid-base disorders. In: Seldin and Giebisch's The Kidney, Fourth Edition: Physiology & Pathophysiology 1-2. RJ Alpern, SC Hepert (eds). Academic Press, 2214-2241.

Baynes RE, Smith G, Mason SE, Barrett E, Barlow BM and Riviere JE, 2008. Pharmacokinetics of melamine in pigs following intravenous administration. Food and Chemical Toxicology, 46, 1196-1200.

Bayrd ED, Stickney JM, Hall BE and Watkins CH, 1952. Clinical observations on the use of triethylenemelamine. Cancer, 5, 336-343.

BfR (Federal Institute for Risk Assessment), 2010a. Comparative results for the migration of melamine into water and acetic acid. Data from the Landesuntersuchungsanstalt für das Gesundheits und Veterinärwesen Sachsen (Dresden, D) and provided to EFSA by Dr Karla Pfaff in January 2010.

BfR (Federal Institute for Risk Assessment), 2010b. Migration of melamine into 3 % acetic acid and foods. Data from the German National Reference Laboratory for Materials in contact with food, provided to EFSA by S. Kaus, Federal Institute for Risk Assessment, Berlin (D), March 2010.

Bradley EL, Boughtflower V, Smith TL, Speck DR and Castle L, 2005. Survey of the migration of melamine and formaldehyde from melamine food contact articles available on the UK market. Food Additives & Contaminants, 22, 597-606.

Brown CA, Jeong KS, Poppenga RH, Puschner B, Miller DM, Ellis AE, Kang KI, Sum S, Cistola AM and Brown SA, 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.

Buur JL, Baynes RE and Riviere JE, 2008. Estimating meat withdrawal times in pigs exposed to melamine contaminated feed using a physiologically based pharmacokinetic model. Regulatory Toxicology and Pharmacology, 51, 324-331.

Cameron MA, Sakhaee K and Moe OW, 2005. Nephrolithiasis in children. Pediatric Nephrology, 20, 1587-1592.

Canciolo R, Bischoff K, Ebel J, Van Winkle T, Goldstein R and Serfilippi L, 2008. Clinocopathologic, 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, 729-737.

Cascieri T, Barbee SJ, Hammond BG, Inoue T, Ishida N, Wheeler AG and Schardein JL, 1983. Absence of teratogenic response in rats with monosodium cyanurate. Toxicologist, 3, 65.

CEC (Commission of the European Communities, food-science and techniques), 2003. Nutrient and energy intakes for the European Community. Reports of the Scientific Committee for Food (thirty-first series), 1993.

Chen JS, 2009a. A worldwide food safety concern in 2008-melamine-contaminated infant formula in China caused urinary tract stone in 290,000 children in China. Chinese Medical Journal, 122, 243-244.

Chen JS, 2009b. What can we learn from the 2008 melamine crisis in China? Biomedical and Environmental Sciences, 22, 109-111.

Chen Y, Yang W, Wang Z, Peng Y, Li B, Zhang L and Gong L, 2010. Deposition of Melamine in Eggs from Laying Hens Exposed to Melamine Contaminated Feed. Journal of Agricultural and Food Chemistry, 58, 3512-3516.

CIAA, CEPE and Empac (Confederation of the food and drink industries of the EU, European Council of the Producers and Importers of Paints, Printing inks & Artists' colours, European Metal Packaging), 2009. Letter sent to EFSA on 18th of November 2009.

Clark R, 1966. Melamine crystalluria in sheep. Journal of the South African Veterinary Medical Association, 37, 349-351.

Clark R, Barratt E and Kellerman J, 1965. A comparison between nitrogen retention from urea, triuret and cyanuric by sheep on a low protein roughage diet. Journal of the South African Veterinary Medical Association, 36, 79.

Cremonezzi DC, Diaz MP, Valentich MA and Eynard AR, 2004. Neoplastic and preneoplastic lesions induced by melamine in rat urothelium are modulated by dietary polyunsaturated fatty acids. Food and Chemical Toxicology, 42, 1999-2007.

Cruywagen CW, Stander MA, Adonis M and Calitz T, 2009. Hot topic: pathway confirmed for the transmission of melamine from feed to c'w's milk. Journal of Dairy Science, 92, 2046-2050.

Ding J, 2009. Childhood urinary stones induced by melamine-tainted formula: how much we know, how much we d'n't know. Kidney International, 75, 780-782.

Dobson RL, Motlagh S, Quijano M, Cambron RT, Baker TR, Pullen AM, Regg BT, Bigalow-Kern AS, Vennard T, Fix A, Reimschuessel R, Overmann G, Shan Y and Daston GP, 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.

Dominguez-Estevez M, Constable A, Mazzatorta P, Renwick AG and Schilter B, 2010. Using urinary solubility data to estimate the level of safety concern of low levels of melamine (MEL) and cyanuric acid (CYA) present simultaneously in infant formulas. Regulatory Toxicology and Pharmacology, in press.

DSM (Department of Regulatory Affairs & Product Safety), 2009. Migration tests performed on melamine-containing laminates. Report provided to EFSA in November 2009 by DSM Department of Regulatory Affairs & Product Safety (Polymers).

EC (European Commission), 1986. Report of the Scientific Committee for Food on certain monomers of other starting substances to be used in the manufacture of plastic materials and articles intended to come into contact with foodstuffs. Seventeenth series. Opinion expressed on 14 December 1984. Available from http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_17.pdf.

EFSA (European Food Safety Authority), 2006a. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to 2,2-bis(4-hydroxyphenyl)propane(Bisphenol A). The EFSA Journal. 428, 1-75.

EFSA (European Food Safety Authority), 2006b. Guidance of the Scientific Committee on a request from EFSA related to Uncertainties in Dietary Exposure Assessment. The EFSA Journal. 438, 1-54.

EFSA (European Food Safety Authority), 2007. EFSA provisional statement on a request form the European Commission related to melamine and structurally related to compounds such as cyanuric acid in protein-rich ingredients used for food and feed. Available froww.efsa.europa.eu/en/scdocs/doc/1047.pdf.

EFSA (European Food Safety Authority), 2008a. Statement of EFSA on risks for public health due to the presences of melamine in infant milk and other milk products in China. The EFSA Journal. 807, 1-10.

EFSA (European Food Safety Authority), 2008b. Guidance Document for the use of the Concise European Food Consumption Database in Exposure Assessment. 17 March 2008. 1-11.

EFSA (European Food Safety Authority), 2009. Guidance of the Scientific Committee on Use of the benchmark dose approach in risk assessment. The EFSA Journal. 1150, 1-72.

EMEP (European Monitoring and Evaluation Programme), 2001. Cyromazine summary report (2).Committee for veterinary medicinal products. European Agency for the Evaluation of medicinal products, veterinary medicines and inspections (EMEA). EMEA/MRL/770/00-FINAL, January 2001. Available froww.emea.europa.eu/pdfs/vet/mrls/077000en.pdf.

Enghardt-Barbieri H, Pearson M and Becker W, 2006. Riksmaten – barn 2003. Livsmedels - och näringsintag bland barn i Sverige. Uppsala: Livsmedelsverket.

FAPAS (Food Analysis Performance Assessment Scheme), 2010. Melamine in chocolate. FAPAS Proficiency test 3023. September-October 2009. J. Croucher. The Food and Environment Research Agency, UK.

Falk Filipsson A. Sand S, Nilsson, J and Victorin K, 2003. The benchmark dose method—review of available models, and recommendations for application in heath risk assessment. Critical Rewiews in Toxicology, 33, 505–542.

Fathallah-Shaykh F and Neiberger R, 2008. Uric acid stones. E-medicine. Available from http://emedicine.medscape.com/article/983759-overview.

FERA (Food and Environment Research Agency), 2010. Comparing the concentrations of melamine, cyanuric acid, ammeline and ammelide migrating from melamineware into 3 % acetic acid and foodstuffs. J. Day and E.L. Bradley (eds.). The Food & Environment Research Agency, York (UK), March 2010.

FFSA (Finnish Food Safety Authority), 2010. Data provided to EFSA in February 2010 by the Finnish Food Safety Authority, Helsinki, Finland.

FhILV (Fraunhofer-Institut für Verfahrenstechnik und Verpackung), 2010. Data supplied to EFSA by Dr R Franz, Freising (D) in February 2010.

Fisk PR, Girling AE and Wildey RJ, 2003. Prioritisation of flame retardants for environmental risk assessment. The Environment Agency (UK) 2003.

FSA (Food Standards Agency), 2006. LC-MS method development for the screening of non-volatile and polar compounds present in paper and board and plastic food contact materials. W. Read, M. Scotter and L. Castle. UK Food Standards Agency report on project A03037. Available from www.food.gov.uk/science/research/researchinfo/contaminantsresearch/contactmaterials/a03prog/a03projlist/a03037.

Gamboa da Costa G, Jacob CC, VonTungeln L, Olson G, Warbritton A, Hattan DG, Reimschuessel R and Beland FA, 2010. Assessment of the nephrotoxicity of a seven-day combined-exposure to melamine and cyanuric acid in F344 rats. Abstract for the Annual SOT meeting. 3rd -10th March 2010, Salt Lake city.

Giebisch H and Windhager E, 2003. Medical Physiology; Saunders, Elsevier. In: WF Boron, EL Boelpaep. 845-860.

Gillespie RS and Stapleton FB, 2004. Nephrolithiasis in children. Pediatrics in Review, 25, 131-139.

Gonzalez J, Puschner B, Perez V, Ferreras MC, Delgado L, Munoz M, Perez C, Reyes LE, Velasco J, Fernandez V and Garcia-Marin JF, 2009. Nephrotoxicosis in Iberian piglets subsequent to exposure to melamine and derivatives in Spain between 2003 and 2006. Journal of Veterinary Diagnostic Investigation, 21, 558-563.

Gossner CM, Schlundt J, Ben Embarek P, Hird S, Lo-Fo-Wong D, Beltran JJ, Teoh KN and Tritscher A, 2009. The melamine incident: implications for international food and feed safety. Environmental Health Perspectives, 117, 1803-1808.

Grases F, Costa-Bauza A, Gomila I, Serra-Trespalle S, Alonso-Sainz F and del Valle JM, 2009. Melamine urinary bladder stone. Urology, 73, 1262-1263.

Guan N, Fan Q, Ding J, Zhao Y, Lu J, Ai Y, Xu G, Zhu S, Yao C, Jiang L, Miao J, Zhang H, Zhao D, Liu X and Yao Y, 2009. Melamine-contaminated powdered formula and urolithiasis in young children. The New England Journal of Medicine, 360, 1067-1074.

Hammond BG, Barbee SJ, Inoue T, Ishida N, Levinskas GJ, Stevens MW, Wheeler AG and Cascieri T, 1986. A review of toxicology studies on cyanurate and its chlorinated derivatives. Environmental Health Perspectives, 69, 287-292.

Hard GC, Flake GP and Sills RC, 2009. Re-evaluation of kidney histopathology from 13-week toxicity and two-year carcinogenicity studies of melamine in the F344 rat: morphologic evidence of retrograde nephropathy. Veterinary Pathology, 46, 1248-1257.

He Y, Jiang GP, Zhao L, Qian JJ, Yang XZ, Li XY, Du LZ and Shu Q, 2009. Ultrasonographic characteristics of urolithiasis in children exposed to melamine-tainted powdered formula. World Journal of Pediatrics, 5, 118-121.

Heck HD and 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.

Hirt RC, Steger JE and Simard GL, 2003. Vapor pressure of 2,4,6-triamino-s-triazine (melamine). Journal of Polymer Science A Polymer Physics, 43, 319-323.

Hodge HC, Panner BJ, Downs WL and Maynard EA, 1965. Toxicity of sodium cyanurate. Toxicology and Applied Pharmacology, 7, 667-674.

Huybrechts I, Matthys C, Pynaert I, De Maeyer M, Bellemans M, De Geeter H and De Henauw S, 2008. Flanders preschool dietary survey: rationale, aims, design, methodology, and population characteristics. Archives of Public Health, 66, 5-25.

IARC (International Agency for Research on Cancer), 1999. Melamine. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 73, 329-338.

IOM (Institute of Medicine ), 1991. National Academy of Sciences. Nutrition during lactation. Washington, DC: National Academy Press.

ISAN (Institute of Food Science and Nutrition ), 2010. Migration of melamine from melamine infant dishware to milk, vegetable soup, and tannin free tea. M. Battaglia and G. Piva. Feed & Food Science and Nutrition Institute, Università Cattolica del Sacro Cuore, Piacenza, Italy. March 2010.

Ishiwata H, Inoue T and Tanimura A, 1986. Migration of melamine and formaldehyde from tableware made of melamine resin. Food Additives & Contaminants, 3, 63-69.

Jia LQ, Shen Y, Wang XM, He LJ, Xin Y and Hu YX, 2009. Ultrasonographic diagnosis of urinary calculus caused by melamine in children. Chinese Medical Journal, 122, 252-256.

JMPR (Joint Meeting of the FAO/WHO Panel of Experts on Pesticide Residues in Food), 1991. Pesticide residues in food – 1990 evaluations. Toxicology. World Health Organization, WHO/PCS/91.47, 1991. Cyromazine. Available from www.inchem.org/documents/jmpr/jmpmono/v90pr06.htm.

JMPR (Joint Meeting of the FAO/WHO Panel of Experts on Pesticide Residues in Food ), 2007 Pesticide residues in food. Available from http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/DOWNLOAD/2007_rep/report2007jmpr.pdf and from http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/2007_eva/Evaluation.pdf

JMPR (Joint Meeting of the FAO/WHO Panel of Experts on Pesticide Residues in Food), 2008. Pesticide residues in food – 2006 evaluations. Part II – Toxicological. World Health Organization, 2008.Available from http://whqlibdoc.who.int/publications/2008/9789241665223_eng.pdf.

Jutzi K, Cook AM and Hutter R, 1982. The degradative pathway of the s-triazine melamine. The steps to ring cleavage. Biochemical Journal, 208, 679-684.

Kersting M, Alexy U, Sichert-Hellert W, Manz F and Schoch G, 1998. Measured consumption of commercial infant food products in German infants: results from the DONALD study. Dortmund Nutritional and Anthropometrical Longitudinally Designed. Journal of Pediatric Gastroenterology and Nutrition, 27, 547-552.

Kim CW, Yun JW, Bae IH, Lee JS, Kang HJ, Joo KM, Jeong HJ, Chung JH, Park YH and Lim KM, 2010. Determination of spatial distribution of melamine-cyanuric acid crystals in rat kidney tissue by histology and imaging matrix-assisted laser desorption/ionization quadrupole time-of-flight mass spectrometry. Chemical Research in Toxicology, 23, 220-227.

Kobayashi T, Okada A, Fujii Y, Niimi K, Hamamoto S, Yasui T, Tozawa K and Kohri K, 2010. The mechanism of renal stone formation and renal failure induced by administration of melamine and cyanuric acid. Urological Research, ahead of print, 9 pp.

Kroke A, Manz F, Kersting M, Remer T, Sichert-Hellert W, Alexy U and Lentze MJ, 2004. The DONALD Study. History, current status and future perspectives. European Journal of Nutrition, 43, 45-54.

Lam CW, Lan L, Che X, Tam S, Wong SS, Chen Y, Jin J, Tao SH, Tang XM, Yuen KY and Tam PK, 2009. Diagnosis and spectrum of melamine-related renal disease: plausible mechanism of stone formation in humans. Clinica Chimica Acta, 402, 150-155.

Lam HS, Ng PC, Chu WC, Wong W, Chan DF, Ho SS, Wong KT, Ahuja AT and Li CK, 2008. Renal screening in children after exposure to low dose melamine in Hong Kong: cross sectional study. BMJ, 337, a2991.

Langman CB, 2009. Melamine, powdered milk, and nephrolithiasis in Chinese infants. The New England Journal of Medicine, 360, 1139-1141.

Larrañaga N, Amiano Etxezarreta P, Gorostiza Garai E, Pérez Díez Y, Bidaurrazaga Van-Dierdonck J, Sarasqueta Eizaguirre C, Arrizabalaga Abasolo JJ, Espada Sáez-Torres M and Méndez Navas I 2006. Encuesta de nutrición 2005: Hábitos alimentarios y estado de salud de la población vasca de 4 a 18 años. Primeros resultados. Vitoria-Gasteiz Servicio Central de Publicaciones del Gobierno Vasco, DL. Available from www.osanet.euskadi.net/r85-13714/es/contenidos/informacion/sanidad_alimentaria/es_1247/adjuntos/DietaSana_c.pdf.

Leclercq C, Arcella D, Piccinelli R, Sette S, Le Donne C and Turrini A, 2009. The Italian National Food Consumption Survey INRAN-SCAI 2005-06: main results in terms of food consumption. Public Health Nutrition, 12, 2504-2532.

Li G, Jiao S, Yin X, Deng Y, Pang X and Wang Y, 2010. The risk of melamine-induced nephrolithiasis in young children starts at a lower intake level than recommended by the WHO. Pediatric Nephrology, 25, 135-141.

Lightner DV, Pantoja CR, Redman RM, Hasson KW and Menon JP, 2009. Case reports of melamine-induced pathology in penaeid shrimp fed adulterated feeds. Diseases of Aquatic Organisms, 86, 107-112.

Linardakis M, Sarri K, Pateraki MS, Sbokos M and Kafatos A, 2008. Sugar-added beverages consumption among kindergarten children of Crete: effects on nutritional status and risk of obesity. BMC Public Health, 8, 279.

Lipschitz WL and Stokey E, 1945. The mode of action of three new diuretic: melamine, adenine and formoguanamine. Journal of Pharmacology and Experimental Therapeutics, 83, 235-249.

Liu G, Li S, Jia J, Yu C, He J and Zhu J, 2009. Pharmacokinetic study of melamine in rhesus monkey after a single oral administration of a tolerable daily intake dose. Regulatory Toxicology and Pharmacology, 56, 193-196.

Lu MB, Yan L, Guo JY, Li Y, Li GP and Ravindran V, 2009. Melamine residues in tissues of broilers fed diets containing graded levels of melamine. Poultry Science, 88, 2167-2170.

Lund KH and Petersen AH, 2002. Migration from kitchen- and tableware made of melamine plastic. FødevareRapport 2002:17. Danish Veterinary and Food Administration, Copenhagen.

Lund KH and Petersen JH, 2006. Migration of formaldehyde and melamine monomers from kitchen- and tableware made of melamine plastic. Food Additives & Contaminants, 23, 948-955.

Lyhne N, Christensen T, Groth MV, Fagt S, Biltoft-Jensen A, Hartkopp H, Hinsch H-J, Matthiessen J, Møller A, Saxholt E and Trolle E, 2005. Dietary habits in Denmark 2000-2002. Main results. Copenhagen: Danish Institute for Food and Veterinary Research, Department of Nutrition.

MacKenzie H, 1965. The effect of different levels of cyanuric acid in sheep in winter. Journal of the South African Veterinary Medical Association, 36, 369-370.

MacKenzie H, 1966. Melamine for sheep. Journal of the South African Veterinary Medical Association, 37, 153-157.

MacKenzie H and vanRensburg I, 1968. Ammelide and ammeline as a non-protein nitrogen in supplements for sheep. Journal of the South African Veterinary Medical Association, 39, 41-45.

MAFF (Ministry of Agriculture, Fisheries and Food), 1996. Food Surveillance Information Sheet number 90. May 1996. UK survey of paper and board food contact materials for residual amine monomers from wet strength agents. Available from http://archive.food.gov.uk/maff/archive/food/infsheet/1996/no90/90amine.htm.

Mandeville JA and Nelson CP, 2009. Pediatric urolithiasis. Current Opinion in Urology, 19, 419-423.

Martin RE, Hizo CB, Ong AM, Alba OM and Ishiwata H, 1992. Release of formaldehyde and melamine from melamine tableware manufactured in Philippines. Journal of Food Protection, 55, 632-635.

Mast RW, Jeffcoat AR, Sadler BM, Kraska RC and Friedman MA, 1983. Metabolism, disposition and excretion of [14C]melamine in male Fischer 344 rats. Food and Chemical Toxicology, 21, 807-810.

Melnick RL, Boorman GA, Haseman JK, Montali RJ and Huff J, 1984. Urolithiasis and bladder carcinogenicity of melamine in rodents. Toxicology and Applied Pharmacology, 72, 292-303.

Mitruka BM, Rawnsley HM and Vadehra BV 1977. Clinical biochemical and hematological reference values in normal experimental animals. Masson Publishing USA, Inc., New York. 152-160.

Newton GL and Utley PR, 1978. Melamine as a dietary nitrogen source for ruminants. Journal of Animal Science, 47, 1338-1344.

NRL-FCM (National Reference Laboratory for Food Contact Materials), 2010. Specific migration of melamine from a coating for a kitchen working bench. Data provided to EFSA in January 2010 by the Portuguese NRL-FCM, The Biotechnology College Packaging Department, Catholic University, Porto, Portugal.

NTP (National Toxicology Programme), 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; Available from http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr245.pdf). National Toxicology Program technical Report Series. 245, 1-171.

Ocké MC, van Rossum CTM, Fransen HP, Buurma EJM, de Boer EJ, Brants HAM, Niekerk EM, van der Laan JD, Drijvers JJMM and Ghameshlou Z, 2008. Dutch National Food Consumption Survey - Young children 2005/2006 (350070001). Bilthoven: National Institute for Public Health and the Environment (RIVM).

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

OECD (Organisation for economic co-operation and development), 2002. SIDS Analysis. UNEP Publications: Melamine. Available from http://www.inchem.org/documents/sids/sids/108781.pdf.

Ogasawara H, Imaida K, Ishiwata H, Toyoda K, Kawanishi T, Uneyama C, Hayashi S, Takahashi M and Hayashi Y, 1995. Urinary bladder carcinogenesis induced by melamine in F344 male rats: correlation between carcinogenicity and urolith formation. Carcinogenesis, 16, 2773-2777.

Osborne CA, Lulich JP, Ulrich LK, Koehler LA, Albasan H, Sauer L and Schubert G, 2009. Melamine and cyanuric acid-induced crystalluria, uroliths, and nephrotoxicity in dogs and cats. Veterinary Clinics of North America. Small Animal Practice, 39, 1-14.

Puschner B, Poppenga RH, Lowenstine LJ, Filigenzi MS and Pesavento PA, 2007. Assessment of melamine and cyanuric acid toxicity in cats. Journal of Veterinary Diagnostic Investigation, 19, 616-624.

Räsänen M, Kronberg-Kippilä C, Ahonen S, Uusitalo L, Kautiainen S, Erkkola M, Veijola R, Knip M, Kaila M and Virtanen SM, 2006. Intake of vitamin D by Finnish children aged 3 months to 3 years in relation to sociodemographic factors. European Journal of Clinical Nutrition, 60, 1317-1322.

Reimschuessel R, Andersen W, Turnipseed S, Karbiwnyk C, Mayer T, Nochetto C, Rummel N and Gieseker C, 2009. Residue depletion of melamine and cyanuric acid in catfish and rainbow trout following oral administration. Journal of Veterinary Pharmacology and Therapeutics, 33, 172-182.

Reimschuessel R, Gieseker CM, Miller RA, Ward J, Boehmer J, Rummel N, Heller DN, Nochetto C, de Alwis GK, Bataller N, Andersen WC, Turnipseed SB, Karbiwnyk CM, Satzger RD, Crowe JB, Wilber NR, Reinhard MK, Roberts JF and Witkowski MR, 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, 8-12.

Reyers F, 2008. Melamine is back - in South Africa and in Milk: A veterinary perspective. Veterinary News, December issue, 13-16.

RIVM-RIKILT (National Institute of Public Health and the Environment - Institute of Food Safety), 2008. Modeling of the transfer of melamine from feed to pig tissues. Advice for the Dutch food and consumer product safety authority; Office for risk assessment by the RIVM-RIKILT front office food safety. Advice dated 12-12-2008. Available from www.vwa.nl/cdlpub/servlet/CDLServlet?p_file_id=36644.

Ruprich J, Dofkova M, Rehurkova I, Slamnenikova E and Resova D, 2006. Individual food consumption - the national study SISP04. Prague: CHFCH NIPH. Available from www.chpr.szu.cz/spotrebapotravin.htm.

Serra-Majem L, Garcia-Closas R, Ribas L, Perez-Rodrigo C and Aranceta J, 2001. Food patterns of Spanish schoolchildren and adolescents: The enKid Study. Public Health Nutr, 4, 1433-1438.

SGL (State General Laboratory ), 2010. Data provided to EFSA by Dr Eleni Ioannou- Kakouri, State General Laboratory, Nicosia, Cyprus, 2010.

Shelton DR, Karns JS, McCarty GW and Durham DR, 1997. Metabolism of Melamine by Klebsiella terragena. Applied and Environment Microbiology, 63, 2832-2835.

Shia J, Mallet C, Young M, Li J, Meng Y and Qi C, 2008. Rapid, specific analysis of melamine contamination in infant formula and liquid milk by UPLC/MS/MS. WATERS application note 2008. Available from http://www.waters.com/waters/library.htm?locale=en_US&cid=514887&lid=10087429&xcid=9186.

Simell O, Niinikoski H, Ronnemaa T, Raitakari OT, Lagstrom H, Laurinen M, Aromaa M, Hakala P, Jula A, Jokinen E, Valimaki I and Viikari J, 2009. Cohort Profile: the STRIP Study (Special Turku Coronary Risk Factor Intervention Project), an Infancy-onset Dietary and Life-style Intervention Trial. International Journal of Epidemiology, 38, 650-655.

Stapleton FB, 1983. Renal uric acid clearance in human neonates. Journal of Pediatrics, 103, 290-294.

Stapleton FB, Linshaw MA, Hassanein K and Gruskin AB, 1978. Uric acid excretion in normal children. Journal of Pediatrics, 92, 911-914.

Sugita M, Ishiwata H and 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.

Sugita T, Ishiwata H and Yoshihira K, 1990. Release of formaldehyde and melamine from tableware made of melamine-formaldehyde resin. Food Additives & Contaminants, 7, 21-27.

Sun N, Shen Y, Sun Q, Li XR, Jia LQ, Zhang GJ, Zhang WP, Chen Z, Fan JF, Jiang YP, Feng DC, Zhang RF, Zhu XY and Xiao HZ, 2009a. Melamine related urinary calculus and acute renal failure in infants. Zhonghua Er Ke Za Zhi - Chinese Journal of Pediatrics, 46, 810-815.

Sun Q, Shen Y, Sun N, Zhang GJ, Chen Z, Fan JF, Jia LQ, Xiao HZ, Li XR and Puschner B, 2009b. Diagnosis, treatment and follow-up of 25 patients with melamine-induced kidney stones

complicated by acute obstructive renal failure in Beijing Children's Hospital. European Journal of Pediatrics, 169, 483-489.

Thiersch J, 1957. Effect of 2,4,6, triamino-s-triazine (TR), 2,4,6 tris (ethyleneimino) s-triazine (TEM) and N, N', N"-triethylene phosphoramide (TEPA) on rat litter in utero. Proc. Soc. Exp. Biol. Med., 94, 36-40.

Thompson ME, Lewin-Smith MR, Kalasinsky VF, Pizzolato KM, Fleetwood ML, McElhaney MR and Johnson TO, 2008. Characterization of melamine-containing and calcium oxalate crystals in three dogs with suspected pet food-induced nephrotoxicosis. Veterinary Pathology, 45, 417-426.

Tittlemier SA, 2010. Methods for the analysis of melamine and related compounds in foods: a review. Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment, 27, 129-145.

TNO (The Netherlands Organisation for Applied Scientific Research), 2010. TNO report AR 10-0389/EHK, March 2010, Determination of the release of melamine from melamine utensils.

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.

Tolleson WH, Diachenko GW, Folmer D, Doell D, D. H and Laurel MD, 2009. Background paper on the chemistry of melamine alone and in combination with related compounds. Prepared for the WHO expert meeting on toxicological and health aspects of melamine and cyanuric acid in collaboration with FAO; Supported by Health Canada, Ottawa, Canada, 1– 4 December 2008. World Health Organization, Geneva.

US FDA (United States Federal and Drug Administration), 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. Available from http://www.cfsan.fda.gov/~dms/melamra.html.

US FDA (United States Federal and Drug Administration), 2008a. Interim Safety and Risk Assessment of Melamine and its Analogues in Food for Humans Silver Spring, MD, United States Department of Health and Human Services, Food and Drug Administration Center for Food Safety and Applied Nutrition. Available from http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Melamine/ucm164522.htm.

US FDA (United States Federal and Drug Administration), 2008b. Update: Interim Safety and Risk Assessment of Melamine and its Analogues in Food for Humans Silver Spring, MD, United States Department of Health and Human Services, Food and Drug Administration Center for Food Safety and Applied Nutrition. Available from http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Melamine/ucm164520.htm.

Wallace K, 1996. Ruminal metabolism of peptides and amino acids. Journal of Nutrition, 126, 1326S-1334S.

Wang IJ, Chen PC and Hwang KC, 2009. Melamine and nephrolithiasis in children in Taiwan. The New England Journal of Medicine, 360, 1157-1158.

Wang Y, Bei W and Wang Q, 1990. Crystal structure of melamine cyanuric acid complex (1:1) trihydrochloride, MCA• 3HCl. Journal of Chemical Crystallography, 20, 79-84.

Wheeler AG, Barbee SJ, Hammond BG, Inoue T, Ishida N, Cascieri T and Schardein JL, 1985. Three generation reproduction study in rats administered cyanurate. Toxicologist, 5, 189.

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

WHO (World Health Organization), 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. Available from http://whqlib.doc.who.int/publications/2004/924166052X.pdf.

WHO (World Health Organization), 2007. Sodium Dichloroisocyanurate in Drinking-water Background document for development of WHO Guidelines for Drinking-Water Quality. Available from http://www.who.int/water_sanitation_health/dwq/chemicals/second_addendum_sodium_dichloroisocyanurate.pdf. 1-15.

WHO (World Health Organization), 2009a. Toxicological and Health aspects of melamine and cyanuric acid. Report of a WHO expert meeting in collaboration with FAO. Supported by Health Canada.

WHO (World Health Organization), 2009b. Full report of the Joint FAO/WHO Expert meeting on chlorine-containing disinfectants used in food production and food processing, held in Ann Arbor, USA, 27-30 May 2008. Available from http://www.fao.org/ag/agn/agns/files/Active%20Chlorine%20Report%20Version%20Final%20December%202009.pdf. 1-276.

WHO/IPCS (World Health Organization/ International Programme on Chemical Safety), 2008. Guidance on Characterizing and Communicating Uncertainty in Exposure Assessment and Guidance on Data Quality in Chemical Exposure Assessment have been published as a two-part publication (Harmonization Project Document No 6).p1-175. Available from http://www.who.int/ipcs/publications/methods/harmonization/exposure_assessment.pdf.

Wongthai P, Boonyawiwat W, Poolpipat T and Moonjit P, 2008. Effects of melamine on frog farms: A veterinary case report. Department of Farm Animal Resources and Production Medicine, Faculty of Veterinary Medicine, Kasetsart University, Kamphaengsaengsaen Campus, Nakorn Pathom, Thailand.

Worzalla JF, Kaiman BD, Johnson BM, Ramirez G and Bryan GT, 1974. Metabolism of hexamethylmelamine-ring-14C in rats and man. Cancer Research, 34, 2669-2674.

Wu YN, 2009a. What have we learned from melamine event. Lecture presented at the Sino-Dutch workshop on food safety risk assessment; National Institute of Nutrition and Food Safety; Chinese Center for Disease Control and Prevention. Beijing, August, 2009.

Wu YN, Zhao YF and Li JG, 2009b. A survey on occurrence of melamine and its analogues in tainted infant formula in China. Biomedical and Environmental Sciences, 22, 95-99.

Xie G, Zheng X, Qi X, Cao Y, Chi Y, Su M, Ni Y, Qiu Y, Liu Y, Li H, Zhao A and Jia W, 2009. Metabonomic evaluation of melamine-induced acute renal toxicity in rats. Journal of Proteome Research, 9, 125-133.

Yang F, Mao Y, Zhang X and Ma Z, 2009. LC-MS/MS method for the determination of melamine in rat plasma: toxicokinetic study in Sprague-Dawley rats. Journal of Separation Science, 32, 2974-2978.

Zhang L, Wu LL, Wang YP, Liu AM, Zou CC and Zhao ZY, 2009. Melamine-contaminated milk products induced urinary tract calculi in children. World Journal of Pediatrics, 5, 31-35.

Zhu SL, Li JH, Chen L, Bao ZX, Zhang LJ, Li JP, Chen JH and Ji KM, 2009. Conservative management of pediatric nephrolithiasis caused by melamine-contaminated milk powder. Pediatrics, 123, e1099-1102.

## APPENDICES

### APPENDIX I- RIVM-RIKILT MODIFICATION OF THE BUUR ET AL. (2008) MODEL FOR MELAMINE IN PIGS

Buur et al. (2008) described a physiologically-based pharmacokinetic modelling (PBPK) model for melamine in pigs. Table 2 in this publication supplies the basic set of differential equations for the mass balance of melamine in the 4 model compartments, i.e. liver, kidney, carcass and plasma.

Figure 3A in Buur et al. (2008) presents a model simulation of the melamine plasma concentration-time course in pigs who have been exposed to 6.13 mg melamine/kg-b.w. via intravenous infusion. As shown and discussed in the paper, the applied model describes the data far from adequately (see Figure 1 in this Appendix). The reason for this is that the model implementation which has been used to generate Figure 1 does not match the model as described in Table 2 of the Buur et al. (2008) paper: it overtly overestimates renal clearance. This becomes clear when the exact mathematical code as presented in Buur et al. (2008) table 2 is implemented to draft Figure.2 (in this Annex), as has been done by RIVM-RIKILT (2008).

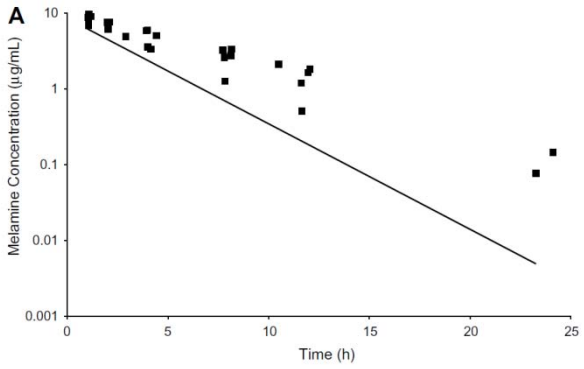



**Figure 1:** Figure as presented by Buur et al., 2008. Comparison of the porcine melamine PBPK model against porcine plasma data. Solid line: model prediction. Squares: observed data (Baynes et al., 2008).

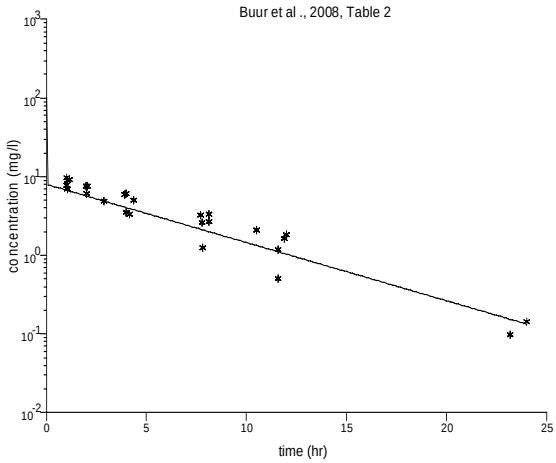



**Figure 2:** Melamine PBPK model against porcine plasma data. Solid line: model output when implemented with the algorithms as presented in Buur et al. (2008) table 2. Squares: observed data (Baynes et al., 2008). The renal clearance is proportional to the arterial plasma concentration.

The respective algorithms have been presented below: first the algorithms of the paper by Buur et al. (2008) are given, and thereafter the algorithms in the RIVM-RIKILT re-implementation are presented.

***Algoritms of the pig melamine PBPK model as published in Buur et al. (2008); table 2:***

| Tissue compartment | Equation |
|---|---|
| Liver | $V_{liver} \cdot \frac{dC_{liver}}{dt} = \left(C_a - \frac{C_{liver}}{P_{liver}}\right) \cdot Q_{liver} + PO_{input}$ |
| Kidney | $V_{kidney} \cdot \frac{dC_{kidney}}{dt} = \left(C_a - \frac{C_{kidney}}{P_{kidney}}\right) \cdot Q_{kidney} - C_a \cdot CL_{renal}$ |
| Carcass | $V_{carcass} \cdot \frac{dC_{carcass}}{dt} = \left(C_a - \frac{C_{carcass}}{P_{carcass}}\right) \cdot Q_{carcass}$ |
| Plasma | $V_{plasma} \cdot \frac{dC_{plasma}}{dt} = \left(Q_{liver} \cdot \frac{C_{liver}}{P_{liver}}\right) + \left(Q_{kidney} \cdot \frac{C_{kidney}}{P_{kidney}}\right) + \left(Q_{carcass} \cdot \frac{C_{carcass}}{P_{carcass}}\right) + IV_{input} - C_{plasma} \cdot Q_{tot}$ |

Subscripts: liver, kidney, carcass, and plasma are tissue blocks. $C$, concentration of melamine in respective tissues or in arterial blood (subscript a); $V$, volume of respective tissues; $Q$, blood flow to respective tissues with $Q_{tot}$ being cardiac output; $P$, tissue:blood partition coefficient for respective tissues; $CL_{renal}$, renal clearance; $IV_{input}$ and $PO_{input}$ intravenous and oral dosing inputs, respectively.

***Relevant part of computer model code as implemented by RIVM-RIKILT to generate Figure 2:***

```
dAldt=DOSEpo+Ql*(Cp-Cl/Pl);

dAkdt=Qk*(Cp-Ck/Pk)-Clren*Cp;

dAcdt=Qc*(Cp-Cc/Pc);

dApdt=-Ql*(Cp-Cl/Pl)-Qk*(Cp-Ck/Pk)-Qc*(Cp-Cc/Pc)+DOSEiv;
```

in which:

dAldt, dAkdt, dAcdt, dApdt = change in melamine amount in liver, kidney, carcass and blood in time interval dt;

Ql, Qk, Qc = bloodflow through liver, kidney and carcass

Cp, Cl, Ck, Cc = melamine concentrations in blood, liver, kidney and carcass

Pl, Pk, Pc = blood-tissue partition coefficients for liver, kidney and carcass

Clren = renal clearance

DOSEpo, DOSEiv = algorithms to model oral or intravenous exposure, respectively.

The most probable explanation for this discrepancy is that Buur et al. (2008) modelled the total renal clearance in proportion to the total kidney tissue concentration, instead of arterial or venous kidney blood. Indeed, when the code is modified to implement this condition, the Figure 3A from Buur et al. (2008) (= Figure 1 in this appendix) could be reproduced. This modified code is given below, and the concentration-time curve resulting from it is represented in Figure 3.

***Modified RIVM code resulting in a concentration-time curve similar to the one shown n Buur et al. (2008) Figure 3A (compare Figure 1 and 3 in this annex.)***

dAldt=DOSEpo+Ql*(Cp-Cl/Pl);

dAkdt=Qk*(Cp-Ck/Pk)-Clren*Ck;

dAcdt=Qc*(Cp-Cc/Pc);

dApdt=-Ql*(Cp-Cl/Pl)-Qk*(Cp-Ck/Pk)-Qc*(Cp-Cc/Pc)+DOSEiv;

(for symbols see description of algorithms of the RIVM-RIKILT model above)

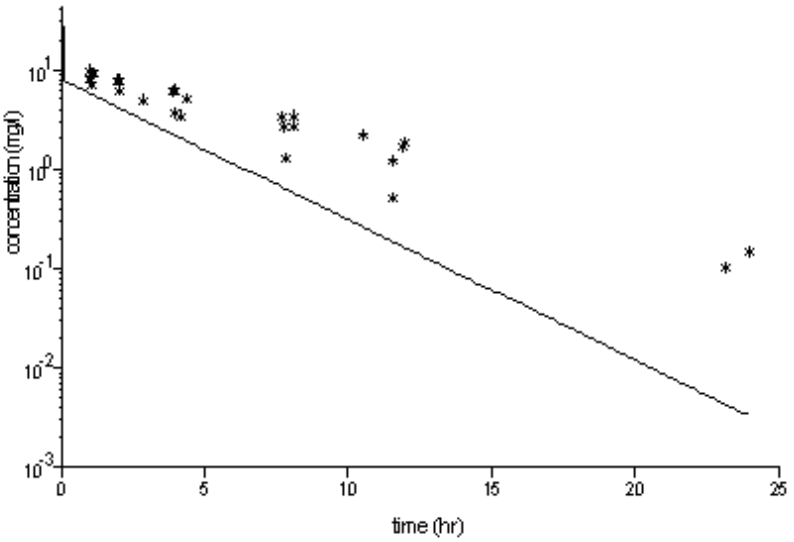



**Figure 3:** The Buur model produces this plasma-concentration time curve when in the second differential equation Ca is replaced by Ck (or in the RIVM code Cp with Ck (= the renal tissue concentration); see the terms with grey highlight.).

**References:**

Baynes RE, Smith G, Mason SE, Barrett E, Barlow BM and Riviere JE, 2008. Pharmacokinetics of melamine in pigs following intravenous administration. Food and Chemical Toxicology, 46, 1196-1200.

Buur JL, Baynes RE and Riviere JE, 2008. Estimating meat withdrawal times in pigs exposed to melamine contaminated feed using a physiologically based pharmacokinetic model. Regulatory Toxicology and Pharmacology, 51, 324-331.

RIVM-RIKILT (National Institute of Public Health and the Environment - Institute of Food Safety), 2008. Modeling of the transfer of melamine from feed to pig tissues. Advice for the Dutch food and consumer product safety authority; Office for risk assessment by the RIVM-RIKILT front office food safety. Advice dated 12-12-2008. Available from www.vwa.nl/cdlpub/servlet/CDLServlet?p_file_id=36644.

**APPENDIX II- BODY WEIGHT, FEED INTAKE AND MELAMINE INTAKE IN MALE RATS FOR THE 90 DAYS MELAMINE (13 WEEKS) STUDIES FROM NTP (1983).** Data provided by the National Center for Toxicological Research (NCTR) of the US Food and Drug Administration (FDA).

**Table 1:** Body weight data (g) in male rats for the 90 days melamine (13 weeks) study from NTP (1983).

| Dose Group (mg/kg diet ) | week 1 | week 2 | week 3 | week 4 | week 5 | week 6 | week 7 | week 8 | week 9 | week 10 | week 11 | week 12 | week 13 | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study I | | | | | | | | | | | | | | |
| 18000 | 120 | 142 | 150 | 168 | 181 | 200 | 200 | 215 | 217 | 243 | 254 | 245 | 255 | 199 |
| 15000 | 121 | 146 | 164 | 177 | 189 | 209 | 220 | 231 | 226 | 250 | 257 | 260 | 259 | 208 |
| 12000 | 116 | 145 | 169 | 183 | 196 | 216 | 226 | 237 | 238 | 258 | 266 | 275 | 276 | 215 |
| 9000 | 121 | 150 | 172 | 192 | 204 | 225 | 237 | 250 | 250 | 266 | 271 | 281 | 288 | 224 |
| 6000 | 117 | 147 | 170 | 192 | 202 | 225 | 238 | 249 | 244 | 261 | 275 | 281 | 287 | 222 |
| Control | 121 | 150 | 173 | 195 | 211 | 235 | 244 | 257 | 260 | 276 | 288 | 292 | 297 | 231 |
| Study II | | | | | | | | | | | | | | |
| 12000 | 143 | 167 | 181 | 197 | 211 | 231 | 243 | 256 | 264 | 269 | 269 | 276 | | 226 |
| 6000 | 148 | 174 | 195 | 212 | 224 | 235 | 249 | 265 | 269 | 276 | 283 | 290 | | 235 |
| 3000 | 150 | 176 | 201 | 218 | 231 | 242 | 255 | 266 | 278 | 287 | 291 | 299 | | 241 |
| 1500 | 148 | 177 | 199 | 221 | 228 | 244 | 257 | 266 | 276 | 286 | 294 | 302 | | 242 |
| 750 | 152 | 183 | 200 | 221 | 238 | 246 | 260 | 271 | 280 | 285 | 294 | 302 | | 244 |
| Control | 153 | 187 | 199 | 228 | 243 | 255 | 267 | 279 | 289 | 302 | 306 | 312 | | 252 |

**Table 2:** Feed consumption data (g per day) in male rats for the 90 days melamine (13 weeks) studies from NTP (1983). Data provided by the National Center for Toxicological Research (NCTR) of the US Food and Drug Administration (FDA).

| Dose Group (mg/kg diet) | week 1 | week 2 | week 3 | week 4 | week 5 | week 6 | week 7 | week 8 | week 9 | week 10 | week 11 | week 12 | week 13 | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study I | | | | | | | | | | | | | | |
| 18000 | 16 | 17 | 15 | 16 | 17 | 19 | 17 | 20 | 20 | 22 | 21 | 18 | 21 | 18 |
| 15000 | 15 | 17 | 17 | 18 | 20 | 20 | 20 | 18 | 20 | 20 | 18 | 20 | 20 | 19 |
| 12000 | 16 | 18 | 17 | 19 | 19 | 20 | 21 | 21 | 22 | 21 | 20 | 21 | 18 | 19 |
| 9000 | 17 | 18 | 20 | 21 | 18 | 24 | 20 | 20 | 21 | 22 | 17 | 22 | 23 | 20 |
| 6000 | 21 | 21 | 17 | 19 | 19 | 22 | 21 | 21 | 20 | 17 | 20 | 20 | 22 | 20 |
| Control | 18 | 20 | 16 | 21 | ne | 23 | 19 | 23 | 22 | 22 | 22 | 20 | 24 | 21 |
| Study II | | | | | | | | | | | | | | |
| 12000 | 16 | 17 | 20 | 23 | 25 | 28 | ne | 25 | 21 | 28 | 19 | 24 | 25 | 23 |
| 6000 | 14 | 17 | 23 | 26 | 23 | 26 | ne | 27 | 20 | 27 | 24 | 15 | ne | 22 |
| 3000 | 15 | 18 | 23 | 26 | 21 | 25 | 22 | 26 | 22 | 28 | 28 | 24 | 26 | 23 |
| 1500 | 15 | 19 | 24 | 23 | 21 | 26 | 21 | 30 | 23 | 27 | 23 | 22 | 24 | 23 |
| 750 | 19 | 23 | 23 | 24 | 24 | 25 | 20 | 24 | 22 | ne | 21 | 24 | 23 | 23 |
| Control | 22 | 26 | 30 | 26 | 30 | 29 | 25 | 27 | 22 | ne | 26 | ne | 26 | 26 |

**Table 3:** Melamine Intake (mg/kg b.w.per day) in male rats for the 90 days melamine (13 weeks) studies from NTP (1983). Data provided by the National Center for Toxicological Research (NCTR) of the US Food and Drug Administration (FDA).

| Dose Group (mg/kg diet) | week 1 | week 2 | week 3 | week 4 | week 5 | week 6 | week 7 | week 8 | week 9 | week 10 | week 11 | week 12 | week 13 | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study I | | | | | | | | | | | | | | |
| 18000 | 2400 | 2155 | 1800 | 1714 | 1691 | 1710 | 1530 | 1674 | 1659 | 1630 | 1488 | 1322 | 1482 | 1712 |
| 15000 | 1860 | 1747 | 1555 | 1525 | 1587 | 1435 | 1364 | 1169 | 1327 | 1200 | 1051 | 1154 | 1158 | 1395 |
| 12000 | 1655 | 1490 | 1207 | 1246 | 1163 | 1111 | 1115 | 1063 | 1109 | 977 | 902 | 916 | 783 | 1134 |
| 9000 | 1264 | 1080 | 1047 | 984 | 794 | 960 | 759 | 720 | 756 | 744 | 565 | 705 | 719 | 854 |
| 6000 | 1077 | 857 | 600 | 594 | 564 | 587 | 529 | 506 | 492 | 391 | 436 | 427 | 460 | 578 |
| 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Study II | | | | | | | | | | | | | | |
| 12000 | 1343 | 1222 | 1326 | 1401 | 1422 | 1455 | | 1172 | 955 | 1249 | 848 | 1043 | | 1221 |
| 6000 | 568 | 586 | 708 | 736 | 616 | 664 | | 611 | 446 | 587 | 509 | 310 | | 576 |
| 3000 | 300 | 307 | 343 | 358 | 273 | 310 | 259 | 293 | 237 | 293 | 289 | 241 | | 292 |
| 1500 | 152 | 161 | 181 | 156 | 138 | 160 | 123 | 169 | 125 | 142 | 117 | 109 | | 144 |
| 750 | 94 | 94 | 86 | 81 | 76 | 76 | 58 | 66 | 59 | | 54 | 60 | | 73 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | 0 |

## References

NTP (National Toxicology Programme), 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; Available from http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr245.pdf). National Toxicology Program technical Report Series. 245, 1-171.

## APPENDIX III - METHODS APPLIED FOR THE BMD ANALYSIS OF ANIMAL DATA OF THE 13 WEEKS NTP (1983) STUDIES IN F344 MALE RATS AND OF HUMAN DATA FROM THE STUDY OF CHILDREN LI ET AL. (2010)

### *Benchmark dose modelling*

The US EPA's benchmark dose software (BMDS) 2.1.1[38] was used for modelling the incidence of melamine induced urolithiasis in male rats and the prevalence of nephrolithasis in young children. The PROAST software was used for cross checking the BMDS results and for a stratified analysis of the two studies reported on male rats from the NTP study.

The models available in the BMDS software used for this analysis, model fitting characteristics and goodness-of-fit statistics, and methods for comparing the models in order to decide which model to use for obtaining the benchmark dose lower confidence limit (BMDL) as reference point are outlined below. The following dose-response models (see Table 3 in EFSA, 2009) were fitted to the exposure-incidence data:

- *Probit*
- *Log-Probit*
- *Logistic*
- *Log-logistic*
- *Weibull*
- *Multistage*
- *Gamma-Multihit*

In addition the *Quantal-Linear* model was also reported in cases where its outcome was different from that of the Weibull model. Note that for constraint modelling the results of the Multistage, Quantal Linear and Gamma often coincide with the outcome of the Weibull model.

$BMD_{10}$ and $BMDL_{10}$ values were calculated for an extra 10 % risk (BMR=0.1). The benchmark response level (BMR) was set equal to 10 % because of the analysis of incidence type data, in accordance with the Scientific Opinion of the EFSA (EFSA, 2009). There has been also stated that "ideally the BMR would reflect an effect size that is negligible or non-adverse" and that "the benchmark dose response (BMR) chosen should not be too small to avoid having to estimate a BMD by extrapolation outside the range of observation". Therefore, the ratio of the lowest dose and the BMDL was calculated for the animal data. For the human data this factor could not be calculated since the low dose data were only available as aggregate data grouped in dose-intervals. The choice of a BMR=10 % addresses, in particular, experimental animal data where the number of individuals per dose group would be usually not larger than n=50, often much smaller.

The exposure levels of the individuals in the epidemiological study of Li et al. (2010) were retrospectively calculated based on reported consumption of formula feeding and breast feeding through a questionnaire. These were non-measured exposure data and they were reported by intervals only, where the control group obviously represents the group of children for whom no formula

[38] http://www.epa.gov/ncea/bmds/about.html

feeding was reported. The lowest exposure interval, different from the control group, ranged from > 0 to 0.2 mg/kg current weight of the child per day. Note that it is not possible for these data to indicate whether the BMD/L value is lower or higher than a lowest exposure level. Unfortunately the authors had provided no further information about the distribution of the individually calculated exposure levels when aggregating the exposure data into exposure intervals. Such information could otherwise have been used to determine a representative dose level of each of those intervals. Note also that the upper end exposure interval aggregated all values (16 children with stones and 31 without) as higher than 51.2 mg/kg current weight of the child per day. The BMD analyses used for that interval the point of 76.8 mg/kg current weight of the child per day, which corresponds to the midpoint of an interval ranging from 51.2 to 102.4. This was thought to be a logical extension of the geometrically increasing sequence of the exposure intervals chosen by these authors. The recruitment of the total of 7181 children analysed by Li et al. (2010) was performed in two steps and therefore the design of that study is not well defined. The 683 children with stones diagnosed by ultrasonography with complete survey information were recruited from 14-24 September 2008 following the Chinese government instituted policy of free screening issued on 12 September 2008. The 6498 children with no stones diagnosed by ultrasonography with complete survey information were recruited from 25 September – 5 October 2008 in the Survey of Children's Health and Feeding Status in Beijing using a sample size of 41 000 taken from a population of 300 000 children of age not larger than 3 years. It is unclear whether the study of 7181 children is a cross-sectional study or a case-control study since no information is available for the remaining 34 502 children of the Survey of Chidren's Health and Feeding Status in Beijing not described in Li et al. (2010) (see also the comment below Table A2).

Use of Constraints. It has been noted in the comments to BMDS 2.0 software (see BMDS 2.0 Help or manual of BMDS2.0/2.1) that unconstraint modelling (e.g. a slope or power parameter allowed to be less than 1) would cause an infinite slope at dose zero in some models. BMDS allows therefore restricting those critical parameters of the dose-response model to avoid an infinite slope at dose zero, For the Log-logistic and Log-Probit model this translates into constraints of the slope parameter, for the Weibull and Gamma model into constraints of the power parameter, and for the Multistage models into constraints of the so-called "beta" parameters. The BMDS Tutorial[39] warns about numerical problems in calculating the confidence interval in models in which dose is raised to a power which is a parameter to be estimated (such as a Weibull model) and therefore recommends to use constraints in that case. This issue was also commented in WHO (2009a) who used exclusively constraint models for deriving a BMDL values. Constraints of model parameters for avoiding infinite slopes of the fitted dose-response curve at dose zero are addressed in EFSA (2009), but without giving further advice. For transparency reasons, both the results of the constraint and the unconstraint fitting were shown for models where that option was available in BMDS software. PROAST was also run with and without constraints.

Model Acceptance. The general principle of the BMD approach adopted by the EFSA is to find all models that are compatible with the data, i. e. those with an accepted fit. In the case of non-nested models, where no exact statistical test criterion can be recommended, an acceptable model should in principle provide a reasonable description of the dose-response data. Therefore, a goodness-of-fit is judged as sufficient if a goodness-of-fit of the model to the data shows a p-value not smaller than 0.1, e. g. using the likelihood ratio test. For the data of this opinion, acceptability of a model was assessed using the log- likelihood value associated with the fitted model tested versus the full model and versus the reduced model in each model fit.

- The full model is the model that does not assume any dose-response function (its parameters are simply the frequencies per dose level). Its log-likelihood is therefore identical for each model fit as long as the same data set us used.

[39] See http://www.epa.gov/ncea/bmds/bmds_training/methodology/intro.htm

- The reduced model is the model with no dose-relationship (it is a straight line parallel to the dose axis representing mean exposure of the total sample). Its log-likelihood is therefore identical for each model fit as long as the same data set us used.

This approach was used (also in the case of non-nested models) such that the fit of the chosen model:

a) should be statistically significantly better than the reduced model ($p<0.05$)

b) should be not significantly worse than the full model ($p\geq0.1$)

for being acceptable. When BMDS software was used the p-values were reported. When PROAST was applied the critical level was set at 0.05.

If there were constraint models which would fulfil the two criteria, the BMD/Ls obtained from them were considered as acceptable models for the risk characterisation.

If none of the constraint models would fulfil the criteria, the set of unconstraint models would be searched for accepted models and BMD/Ls obtained from them would be considered as acceptable models for the risk characterisation. That was the case for the human data of Li et al. (2010).

If none of the constraint as well as none of the unconstraint models would obey the criterion b) above the acceptance boundary for the p-value could be reduced from 0.1 to a lower value and the procedure described above would be applied with that boundary. This specific case did however not occur in this analysis.

The Akaike information criterion (AIC) has also been used as an approximate criterion for comparing the fits of non-nested models (Falk Filipsson et al., 2003). Note that the AIC is not recommended for use in the EFSA Opinion on the BMD. Therefore, it was used as supportive criterion only. Furthermore, the overall p-value of the chi-square goodness-of-fit is calculated based on the residuals describing the difference between estimated and observed frequencies. Note, that the lower the *chi-square* value and the higher the calculated p-value to reject the model the better the fit. All relevant statistics for the suitability of the fit as provided by the BMDS software were reported. Consistency in the outcome of those criteria supports confidence for having chosen the best model. Inconsistency is a sign of enlarged uncertainty of model fitting and consequently of uncertainty of the reported BMD/BMDL values and the chosen reference point.

If a range of models can be accepted, the lowest BMDL of that range is chosen as reference point, according to EFSA (2009). The range of BMDL values from different accepted models was examined wehter it exceeds one order of magnitude.

The $BMD_{10}$ and $BMDL_{10}$ values, as well as the associated statistics for the models used, were presented in tables A1-A4.

**References**

EFSA (European Food Safety Authority), 2009. Guidance of the Scientific Committee on Use of the benchmark dose approach in risk assessment. The EFSA Journal. 1150, 1-72.

Falk Filipsson A. Sand S, Nilsson, J and Victorin K, 2003. The benchmark dose method—review of available models, and recommendations for application in heath risk assessment. Critical Rewiews in Toxicology, 33, 505–542.

Li G, Jiao S, Yin X, Deng Y, Pang X and Wang Y, 2010. The risk of melamine-induced nephrolithiasis in young children starts at a lower intake level than recommended by the WHO. Pediatric Nephrology, 25, 135-141.

## APPENDIX IV- BMD ANALYSIS OF FOR THE 13 WEEKS MELAMINE STUDIES OF THE NTP (1983) IN F344 MALE RATS

**Table A1.1:** $BMD_{10}$ and $BMDL_{10}$ calculations for the second 13 weeks NTP study **(Low Dose 13 Weeks)** on F344 male rats with mean intake during study as dose metric. Consumption was measured during the study based on administered dietary intake related to mean body weight obtained by weekly weighing, see Appendix II. The lowest dose group was that of 73 mg/kg b.w per day with 2/10 incidence; the next higher doses were 144, 292, 567, 1221 mg/kg b.w. per day with incidences 5/10, 7/10, 9/10 and 9/9, respectively; the control group showed 1/10 incidence (see Table 37).

| Model | BMR Extra risk (%) | Nd, Nf | Log- likelihood | p-value | Accepted with p≥0.1 | BMD[1] | BMDL[1] |
|---|---|---|---|---|---|---|---|
| Full model | | | 24.6 | | | | |
| Reduced model | | | 40.5 | | | | |
| Constraint Modelling | | | | | | | |
| Probit | 10 | 6,2 | 25.4 | 0.78 | yes | 65.7 | 47.1 |
| Log-Probit | 10 | 6,3 | 24.8 | 0.93 | yes | 61.9 | 33.2 |
| Logistic | 10 | 6,2 | 25.3 | 0.81 | yes | 66.3 | 45.6 |
| Log-Logistic | 10 | 6,3 | 24.9 | 0.88 | yes | 60.7 | 19.0 |
| Weibull | 10 | 6,3 | 24.8 | 0.92 | yes | 41.1 | 19.2 |
| Gamma | 10 | 6,3 | 24.8 | 0.92 | yes | 45.3 | 19.2 |
| Multi-stage | 10 | 6,3 | 24.8 | 0.90 | yes | 33.7 | 19.1 |
| Quantal-linear | 10 | 6,2 | 24.9 | 0.94 | yes | 27.8 | 18.8 |
| Unconstraint Modelling | | | | | | | |
| Log-Probit | 10 | 6,3 | 24.8 | 0.93 | yes | 61.9 | 20.6 |
| Log-Logistic | 10 | 6,3 | 24.9 | 0.88 | yes | 60.7 | 19.0 |
| Weibull* | 10 | 6,3 | 24.8 | 0.92 | yes | 41.1 | 8.2 |
| Gamma* | 10 | 6,3 | 24.8 | 0.92 | yes | 45.3 | 5.3 |
| Multi-stage | 10 | 6,3 | 24.8 | 0.90 | yes | 35.7 | 17.6 |

BMR: benchmark response; Nd: number of doses, Nf: number of fitted model parameters; p-value: probability value; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit;[1]mg/kg b.w. per day; * BMDL values were not considered because the fit of the distribution allows infinite slope at dose zero.

**Statistical Results and Result of Model Selection**

All constraint and all unconstraint models were accepted at $p \geq 0.1$ when using the log-likelihood test. The goodness of fit of all constraint models was very good and comparable (p-values of the log-likelihood criterion ranged between 0.78 (probit) and 0.94 (quantal linear). The goodness of fit of the unconstraint models were identical.

The range of the BMD values of the constraint models (27.8-66.3- mg/kg b.w. per day) was comparable with that of the unconstraint models (33.7- 61.9 mg/kg b.w. per day). In fact, the BMD values of the unconstraint models available in BMDS were identical to the BMD values obtained in the constraint fit. The range of the BMDL values of the constraint models (18.8- 47.1 mg/kg b.w. per day) were higher when compared with the unconstraint models (5.3 – 20.6 mg/kg b.w. per day). Except for the log-logistic model, BMDLs of the unconstraint models are lower when using the profile likelihood confidence intervals.

The $BMDL_{10}$s of the accepted models were lower than the lowest dose by a factor of 1.6 – 3.9, except the $BMDL_{10}$s of the unconstraint Weibull and Gamma models which were lower than the lowest dose by a factor of 9.1 and 13.8, respectively.

The BMDLs of the unconstraint Weibull and the unconstraint Gamma model were not considered for deriving a BMDL because of concerns on the validity of the outcome of the profile likelihood confidence limit calculation when allowing infinite slope at dose 0, e.g. indicated by the large difference between the BMDL estimate and the lowest observed dose.

For risk characterisation the Panel identified a $BMDL_{10}$ value of 19 mg/kg b.w. per day, the lowest BMDL obtained by the constraint models.

The graphs of the constraint log-logistic, Weibull and log-probit model fit for the Low Dose NTP 13 weeks study in male rats are shown below

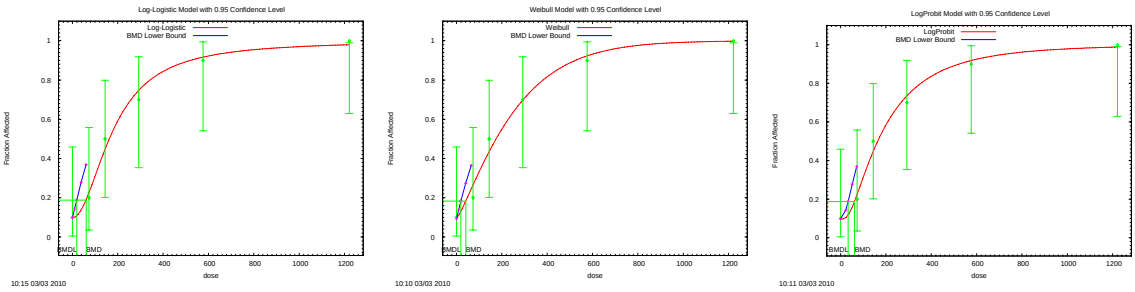


**Table A1.2:** $BMD_{10}$ and $BMDL_{10}$ calculations for the second 13 weeks NTP study **(High Dose 13 Weeks)** on F344 male rats with mean intake during study as dose metric. Consumption was measured during the study based on administered dietary intake related to mean body weight obtained by weekly weighing, see Appendix II. The lowest dose group was 578 mg/kg b.w. per day with 6/12 incidence; the next higher doses were 854, 1134, 1395, 1712 mg/kg b.w. per day with incidences 8/12, 12/12, 10/12 and 12/12, respectively; the control group showed 0/12 incidence (see Table 37).

| Model | BMR Extra risk (%) | Nd, Nf | Log- likelihood | p-value | Accepted with p≥0.1 | BMD[1] | BMDL[1] |
|---|---|---|---|---|---|---|---|
| Full model | | | 21.4 | | | | |
| Reduced model | | | 45.8 | | | | |
| Constraint Modelling | | | | | | | |
| Probit | 10 | 6,2 | 25.4 | 0.09 | no | 233 | 51 |
| Log-Probit | 10 | 6,2 | 24.2 | 0.22 | yes | 288 | 100 |
| Logistic | 10 | 6,2 | 25.3 | 0.099 | no | 254 | 158 |
| Log-Logistic | 10 | 6,2 | 24.3 | 0.21 | yes | 296 | 93 |
| Weibull | 10 | 6,2 | 24.2 | 0.22 | yes | 174 | 52 |
| Gamma | 10 | 6,2 | 24.2 | 0.22 | yes | 220 | 53 |
| Multi-stage | 10 | 6,2 | 24.3 | 0.22 | yes | 132 | 52 |
| Quantal-linear | 10 | 6,1 | 24.9 | 0.22 | yes | 64 | 48 |
| Unconstraint Modelling* | | | | | | | |
| Log-Probit | 10 | 6,2 | 24.2 | 0.22 | yes | 288 | 87 |
| Log-Logistic | 10 | 6,2 | 24.3 | 0.21 | yes | 296 | 93 |
| Weibull | 10 | 6,2 | 24.2 | 0.22 | yes | 174 | 28 |
| Multi-stage | 10 | 6,2 | 24.3 | 0.22 | yes | 132 | 49 |

BMR: benchmark response; Nd: number of doses, Nf: number of fitted model parameters; p-value: probability value; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit;[1]mg/kg b.w. per day; *No fit was possible for the unconstraint Gamma model.

**Statistical Results and Result of Model Selection**

Except two, all constraint models were accepted at $p \geq 0.1$ when using the log-likelihood test. All unconstraint models were acceptable at $p \geq 0.1$. The fit of the unconstraint Gamma model failed.

The goodness of fit of all constraint models was fair and comparable (p-values of the log-likelihood criterion ranged between 0.09 (probit) and 0.22 (quantal linear).

The range of the BMD values of the accepted constraint models (296-64 mg/kg b.w. per day) was comparable with that of the unconstraint models (132 – 288 mg/kg b.w. per day). In fact, the BMD values of the accepted unconstraint models available in BMDS were identical to the BMD values obtained with the constraint fit.

The range of the BMDL values of the constraint models (48 – 158 mg/kg b.w. per day) was higher compared with that of the unconstraint models (28 – 93 mg/kg b.w. per day). Except for the log-logistic model, BMDLs of the unconstraint models are lower when using the profile likelihood confidence intervals. The lowest BMDL was 48 mg/kg b.w. per day.

The $BMDL_{10}$s of the accepted models were lower than the lowest dose by a factor of 5.8 – 11.9, except the $BMDL_{10}$ of the unconstraint Weibull which was lower than the lowest dose by a factor of 20.4.

The constraint models fitted the data almost identically as the unconstraint models.

**For risk characterisation the Panel did not use the high dose 13 weeks study.**

**Table A1.3:** $BMD_{10}$ and $BMDL_{10}$ calculations for the 13 weeks NTP studies **(High Dose 13 Week and Low Dose 13 Week Combined)** on F344 male rats with mean intake during study as dose metric. Consumption was measured during the study based on administered dietary intake related to mean body weight obtained by weekly weighing, see Appendix II. The lowest dose group was that of 73 mg/kg b.w. per day with 2/10 incidence; the higher doses were 144, 292, 567, 1221 mg/kg b.w. per day with incidences 5/10, 7/10, 9/10 and 9/9, respectively, and 854, 1134, 1395, 1712 mg/kg b.w. per day with incidences 8/12, 12/12, 10/12 and 12/12, respectively. The two control groups showed 1/10 and 0/12 incidence, respectively (see Table 37).

| Model | BMR Extra risk (%) | Nd, Nf | Log- likelihood | p-value | Accepted with p≥0.1/0.01 | BMD[1] | BMDL[1] |
|---|---|---|---|---|---|---|---|
| Full model | | | 46.7 | | | | |
| Reduced model | | | 87.1 | | | | |
| Constraint Modelling | | | | | | | |
| Probit | 10 | 11,2 | 58.5 | 0.005 | no/no | 130.4 | 105.8 |
| Log-Probit | 10 | 11,2 | 55.8 | 0.034 | no/yes | 82.7 | 61.5 |
| Logistic | 10 | 11,2 | 58.2 | 0.006 | no/no | 128.8 | 100.9 |
| Log-Logistic | 10 | 11,3 | 54.8 | 0.042 | no/yes | 35.3 | 14.6 |
| Weibull* | 10 | 11,2 | 55.1 | 0.054 | no/yes | 50.6 | 39.6 |
| Unconstraint Modelling | | | | | | | |
| Log-Probit | 10 | 11,3 | 54.6 | 0.047 | no/yes | 38.4 | 9.1 |
| Log-Logistic | 10 | 11,3 | 54.7 | 0.042 | no/yes | 35.3 | 7.9 |
| Weibull | 10 | 11,3 | 54.3 | 0.055 | no/yes | 20.6 | 2.7 |
| Gamma | 10 | 11,3 | 54.3 | 0.057 | no/yes | 15.5 | 0.63 |
| Multi-stage | 10 | 11,3 | 54.8 | 0.040 | no/yes | 42.0 | 28.4 |

BMR: benchmark response; Nd: number of doses, Nf: Number of fitted model parameters; p-value: probability value; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit;[1]mg/kg b.w. per day;

* The following models resulted in identical fits:Weibull=Gamma= mulstistage+Quantal linear , only the Weibull model is presented.

**Statistical Results and Result of Model Selection**

None of the models (constraint and unconstraint) was accepted at $p \geq 0.1$ when using the log-likelihood test. None of the unconstraint models was accepted at $p \geq 0.1$ when using the log-likelihood test.

Three constraint models were acceptedat $p \geq 0.01$: Log-probit, Log-logistic, and Weibull. All unconstraint models were accepted at $p \geq 0.01$.

The goodness of fit of the models accepted by this criterion was limited due to the relative high variability of the incidences at higher doses, but comparable.

The range of the BMD values of the accepted constraint models (33-3 – 82.7 mg/kg b.w. per day) was at higher doses than that of the acceptable unconstraint models (15.5 – 42.0) mg/kg b.w. per day).

The BMDL values of the accepted constraint models 14.6 – 61.5- mg/kg b.w. per day) were higher than those of the accepted unconstraint models (0.63 – 28.4 mg/kg b.w. per day).

The $BMDL_{10}$s of the accepted constraint models were lower than the lowest dose by a factor of 1.1-4.9, whereas those of the unconstraint models were lower by a factor of 2.6-116.

The accepted constraint models fitted the data almost identically as the accepted unconstraint models.

**For risk characterisation the Panel did not use the high dose 13 week and low dose 13 week combined data.**

**Table A1.4:** $BMD_{10}$ and $BMDL_{10}$ calculations for the 13 weeks NTP studies (High Dose 13 Week and Low Dose 13 Week Data Combined in an Analysis where the fit was stratified for study applying PROAST software) on F344 male rats with mean intake during study as dose metric. Consumption was measured during the study based on administered dietary intake related to mean body weight obtained by weekly weighing, see Appendix II. The lowest dose group was that of 73 mg/kg b.w. per day with 2/10 incidence; the higher doses were 144, 292, 567, 1221 mg/kg b.w. per day with incidences 5/10, 7/10, 9/10 and 9/9, respectively, and 854, 1134, 1395, 1712 mg/kg b.w. per day with incidences 8/12, 12/12, 10/12 and 12/12, respectively. The two control groups showed 1/10 and 0/12 incidence, respectively (see Table 37).

| Model | BMR Extra risk (%) | Nd, Nf | Log- likelihood | Accepted with p≥0.05* | BMD[1] | BMDL[1] |
|---|---|---|---|---|---|---|
| Full model | | | 45.9 | | | |
| Reduced model | | | 87.1 | | | |
| Constraint Modelling | | | | | | |
| Probit | 10 | 12,3 | 51.6 | yes | -** | - |
| Log-Probit | 10 | 12,4 | 50.4 | yes | 65.3 | 31.2 |
| Logistic | 10 | 12,3 | 51.3 | yes | 73.1 | 51.4 |
| Log-Logistic | 10 | 12,4 | 54.7 | yes | 65.7 | 30.7 |
| Weibull* | 10 | 12,4 | 50.2 | yes | 45.1 | 19.7 |
| Gamma | 10 | 12,4 | 50.2 | yes | 49.3 | 19.6 |
| Unconstraint Modelling | | | | | | |
| Log-Probit | 10 | 12,4 | 50.4 | yes | 288 | 31.2 |
| Log-Logistic | 10 | 12,4 | 50.6 | yes | 296 | 30.7 |
| Weibull | 10 | 12,4 | 50.2 | yes | 174 | 15.4 |
| Gamma | 10 | 12,4 | 50.2 | yes | 132 | 12.5 |

BMR: benchmark response; Nd: number of doses, Nf: Number of fitted model parameters; p-value: probability value; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit;[1]mg/kg b.w. per day; *PROAST software default value p≥0.05 was used to check for acceptability. **No values provided by the PROAST software.

**Statistical Results and Result of Model Selection**

All constraint and all unconstraint models were accepted at $p \geq 0.05$ when using the log-likelihood test. The goodness of fit of all constraint models was very good and comparable with that of the unconstraint models.

The range of the BMD values of the accepted unconstraint models ranging between 45.1 and 65.7 mg/kg b.w. per day were identical to the BMD values of their constraint counterparts.

The BMDL values of the accepted constraint models ranged between 19.6 and 51.5 mg/kg b.w. per day and were higher than those of the accepted unconstraint models which ranged between 12.5 and 31.2 mg/kg b.w. per day.

The $BMDL_{10}$s of the accepted constraint models were lower than the lowest dose by a factor of 1.4-3.7, whereas those of the unconstraint models were lower by a factor of 2.4 – 5.8.

**For risk characterisation, the Panel did not use the high dose 13 week and low dose 13 week data combined in a stratified analysis using the study type (low /high dose) as covariate.**

**Table A2:** $BMD_{10}$ and $BMDL_{10}$ calculations for the prevalence of nephrolithiasis as reported in the human study of Li et al. (2010).

BMD analysis was based on two populations with a total of 7181 children among whom 683 (9.5 %) had stones and 6498 (90.5 %) had no stone, all diagnosed by ultrasonography and with complete survey information. The BMD analysis assumes that only this subsample of the target sample of 41 000 children of the Survey of Children's Health and Feeding Status in Beijing in a target area of 300 000 children was examined clinically for stones with ultrasonography. Prevalence of nephrolithiasis was related to melamine concentration determined in mg/kg b.w. per day when using current weight of the child. A sequence of dose intervals of geometrically increasing width was used starting with a lowest dose interval ranging from measurable exposure (no LOD/LOQ reported) up to 0.2 mg/kg b.w. per day. That lowest interval group showed an incidence of 97/1334 and the no exposure group an incidence of 11/3062 (see Table 40).

| Model | BMR Extra risk (%) | Nd, Nf | Log- likelihood | p-value | Accepted with p≥0.1 | BMD[1] | BMDL[1] |
|---|---|---|---|---|---|---|---|
| Full model | | | 2069.9 | | | | |
| Reduced model | | | 2256.3 | | | | |
| Constraint Modelling | | | | | | | |
| Probit | 10 | 11,2 | 2165.0 | $<10^{-37}$ | no | 20.2 | 18.2 |
| Log-Probit | 10 | 11,2 | 2169.0 | $<10^{-39}$ | no | 16.9 | 15.0 |
| Logistic | 10 | 11,2 | 2169.7 | $<10^{-39}$ | no | 22.0 | 19.8 |
| Log-Logistic | 10 | 11,3 | 2135.2 | $<10^{-25}$ | no | 9.1 | 7.7 |
| Weibull* | 10 | 11,2 | 2140.3 | $<10^{-27}$ | no | 11.0 | 9.5 |
| Unconstraint Modelling | | | | | | | |
| Log-Probit | 10 | 11,3 | 2076.1 | 0.13 | yes | 1.19 | 0.79 |
| Log-Logistic | 10 | 11,3 | 2076.0 | 0.15 | yes | 1.10 | 0.74 |
| Weibull | 10 | 11,3 | 2076.1 | 0.14 | yes | 1.23 | 0.81 |
| Gamma | 10 | 11,3 | 2076.1 | 0.13 | yes | 1.28 | 0.84 |
| Multi-stage | 10 | 11,3 | 2128.6 | $<10^{-22}$ | no | 7.15 | 6.02 |

BMR: benchmark response; Nd: number of doses, Nf: Number of fitted model parameters; p-value: probability value; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit;[1]mg/kg b.w per day;

* The following models resulted in identical fits:Weibull=Gamma= mulstistage+Quantal linear , only the Weibull model is presented.

**Statistical Results and Result of Model Selection**

None of the constraint models is accepted at $p \geq 0.1$ (nor at $p \geq 0.01$).

Four of the constraint models are accepted at $p \geq 0.1$: Log-logistic, Log-probit, Weibull and Gamma.

The BMDL values of the accepted constraint models ranged between 1.10 and 1.28 mg/kg b.w. per day.

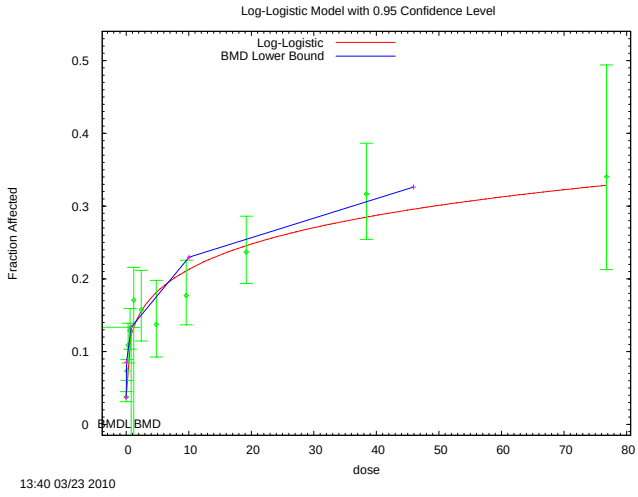
The $BMDL_{10}$s of the accepted constraint models ranged between 0.84 and 0.74 mg/kg b.w. per day.

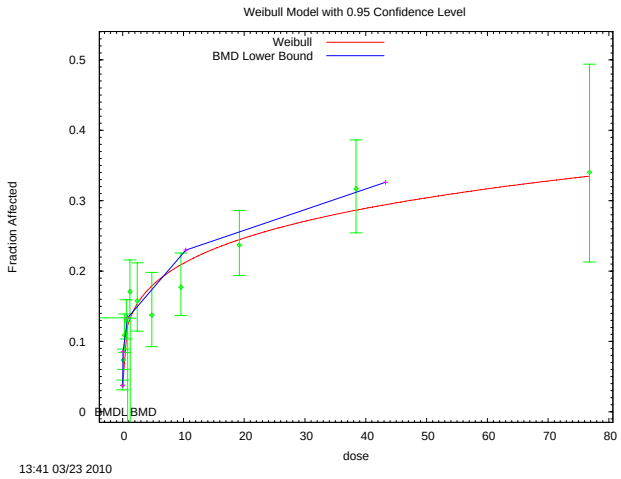
The $BMDL_{10}$s of the accepted constraint models were higher than the lowest exposure group.

**The Panel did not use the data of the Li et al. (2010) study for the derivation of a reference point because of uncertainties in the sampling procedure as well as in exposure estimation.**

Assumptions of this analysis are summarized below. This analysis is based on a prevalence of nephrolithiasis of 9.5 %. If that incidence were to be lower because of a different sample size to that assumed, here, then the BMD/L values would accordingly be higher.

The graphs of the unconstraint log-logistic and Weibull model are shown below.




**Assumption used for the BMD analysis of the data of Li et al. (2010) as reported in Table A2.**

The data of Table 40 are based on two sources:

A) A population of 1788 children diagnosed by ultrasonography with urinary stones from 14 to 24 of September 2008 in hospitals in Beijing and reported to the Beijing Municpal Health Bureau. That population was examined by a questionnaire and the children revisited the hospitals during 25-30 September 2008. Only cases with definitive imaging were reported. A total of 683 children of that population was included into the study according to the study protocol of Li et al. (2010). Those were not older than 3 years and had provided complete survey information.

B) A population from a survey taken between 25 September and 5 October 2008 from a so called target sample of size 41 000 in Beijing (Survey of Children's Health in Beijing). Those children were no more than 3 years old. The sample was subdivided into 8 districts of 2000 children (16 000) and 10 districts of 2500 children (25 000). Within these subsamples clusters of 100 to 200 children were formed according to subdistrict or township. This survey planned for 41000 children covered an area of a total of 300 000 children and was conducted with the participation of 36 centers. The size of the collected sample was 39729 children. Li et al. (2010) report in Figure 1 that in the collected sample were 7 064 children diagnosed by ultrasonography without stones. Among them were 6 498 children with complete survey information.

The BMD Analysis combined the two data sets into one of children who were diagnosed by ultrasonography for the presence or absence of stones in the area of Beijing and in the time period of the first investigations starting in September 2008. This analysis is based on the assumption that the sample of 7108 children reported in Table 2 of Li et al. (2010) can be used as a random sample out of the population of children of not older than 3 years. This requires in particular the following:

1) The two populations of size 1788 and 41 000 are comparable and randomly sampled out of the described area of Beijing.

2) The loss of 252 investigated children diagnosed by ultrasonography with urinary stones of age larger than 3 years in population B does not affect the comparison of the two populations.

3) The loss of 541 children diagnosed by ultrasonography with urinary stones and not investigated in the population of A, and the loss of 32 665 children who were collected but not diagnosed by ultrasonography, according to the information given by Li et al. (2010), are comparable. This assumes, in particular, that only 7 064 children of the target population of 41 000 were diagnosed by a valid clinical method.

4) The BMD analysis has taken the same assumptions as in the analysis carried out by Li et al. (2010). No children were diagnosed with stones by ultrasonography in the collected sample of 39 729. If such children would have been diagnosed with stones by ultrasonography, they would have been already part of population A.

5) There was no risk based application of ultrasonography in the whole population of 42 788 children.

Note: the validity of the assumptions can not be assessed with the information available in the publication. Given these assumptions, the BMD analysis was based on a total of 7 181 children among which 683 (9.5 %) had stones. This assumes a prevalence of stones in the investigated population of the whole population of the target area, namely about 3900 children with stones among the 41 000 children or 28 500 children with stones in the Beijing area. Only if those assumptions hold, the BMD analysis as done in this opinion is valid. Alternatively, one would have to relate the 683 children diagnosed by ultrasonography with stones not to 7181 but to a larger number with the consequence of a lower incidence. When related to 41 000; the incidence in the population would be 1.7 %, when related to 300 000 it would be 0.23 %. When using the data of Li et al. (2010), a prevalence of stones of 9.5 % would give BMDL values for the log-logistic, log probit and Weibull model of 0.00016, 0.00070, and 0.00010 mg/kg b.w. per day whereas for a prevalence of 1.7 % and 0.23 % respective BMDLs would be of 0.10, 0.11, 0.10 and 54.1, 60.1 and 60 mg/kg b.w. per day for the three models.

## References

NTP (National Toxicology Programme), 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; Available from http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr245.pdf). National Toxicology Program technical Report Series. 245, 1-171.

Li G, Jiao S, Yin X, Deng Y, Pang X and Wang Y, 2010. The risk of melamine-induced nephrolithiasis in young children starts at a lower intake level than recommended by the WHO. Pediatric Nephrology, 25, 135-141.

**ABBREVIATIONS**

| | |
|---|---|
| AIC | The Akaike information criterion |
| AQSIQ | China's General Administration of Quality, Supervision, Inspection and the Quarantine |
| BMD | Benchmark dose |
| BMDL | Benchmark dose lower confidence limit |
| BMDS | Benchmark dose software |
| BMR | Benchmark response level |
| BPA | Bisphenol A |
| b.w. | Body weight |
| CEF | Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids |
| CEPE | European Council of the Producers and Importers of Paints, Printing Inks & Artists' colours |
| CI | Confidence interval |
| CIAA | Confederation of the food and drink industries of the EU |
| Clren | Renal clearance |
| $C_{max}$ | Maximum concentration |
| CONTAM Panel | Panel on Contaminants in the Food Chain |
| DAD | Diode array detection |
| EC | European Commission |
| EFSA | European Food Safety Authority |
| ELISA | Enzyme-linked immunosorbent assay |
| Empac | European Metal Packaging |
| EPA | U.S. Environmental Protection Agency |
| ESR | Existing Substances Regulation |
| EU | European Union |
| FAPAS | Food analysis performace assessment scheme |
| FCM | Food contact materials |
| FDA | Food and Drug Administration |
| FhILV | Fraunhofer-Institut für Verfahrenstechnik und Verpackung |
| FTIR | Scanning electron microscopy and infrared spectroscopy |
| GC | Gas chromatography |
| GC-MS | Gas chromatography coupled to mass spectrometry |
| GI | Gastrointestinal |
| HCl | Hydrochloric acid |
| HPLC-UV | High-performance liquid chromatography – ultraviolet |
| IARC | International Agency for Research on Cancer |
| IOM | Institute of Medicine |
| i.p. | intraperitoneal |
| ISAN | Institute of Food Science and Nutrition |
| i.v. | Intravenous |
| JIG | Joint industry group |
| JMPR | Joint FAO/WHO Meetings on Pesticide Residues |
| JRC | Joint research Centre |
| Ka | Rate of gastric absorption |
| Kd | Dissociation constant |
| Kst | Rate of gastric emptying |
| LB | Lower bound |
| LC-MS | Liquid chromatography coupled to mass spectrometry |
| LC-MS/MS | Liquid chromatography coupled to tandem mass spectrometry |
| LC-UV | Liquid chromatography ultraviolet |
| $LD_{50}$ | Lethal dose – the dose required to kill half the members of a tested animal population |

| | |
|---|---|
| LOAEL | Lowest-observed-adverse-effect level |
| LOD | Limit of detection |
| LOQ | Limit of quantification |
| MAFF | Ministry of Agriculture, Fisheries and Food |
| MCA | Melamine – cyanuric acid |
| MRl | Maximum Residue Limits |
| M-UA | Melamine – uric acid |
| NCTR | National Center for Toxicological Research |
| Nd | Number of doses |
| Nf | Number of fitted model parameters |
| NMR | Nuclear magnetic resonance |
| NOAEL | No-observed-adverse-effect level |
| NOEL | No-observed-effect level |
| NPN | Non-protein nitrogen |
| NRL | National Reference Laboratory |
| NTP | National Toxicology Programme |
| OECD | Organisation for Economic Co-operation and Development |
| OR | Odds ratio |
| P95 | $95^{th}$ percentile |
| PBPK | Physiologically-based pharmacokinetic modelling |
| p.o. | per os |
| p-value | Probability value |
| RASFF | Rapid Alert System for Food and Feed |
| REACH | Registration, Evaluation, Authorisation and Restriction of Chemicals |
| RIVM-RIKILT | National Institute of Public Health and the Environment – Institute of Food Safety, The Netherlands |
| SAF | Sampling adjustment factor |
| SCF | Scientific Committee for Food |
| SD | Standard deviation |
| SGL | State General Laboratory |
| SML | Specific migration limit |
| S/V | Surface to volume ratio |
| $T_{max}$ | Time to maximum concentration |
| TDI | tolerable daily intake |
| TNO | The Netherlands Organisation for Applied Scientific Research |
| UA | Concentration of free uric acid in urine |
| UB | Upper bound |
| USA | United States of America |
| WHO/IPCS | World Health Organization/ International Programme on Chemical Safety |