U.S. Food and Drug Administration
Department of Health and Human Services
CENTER FOR FOOD SAFETY AND APPLIED NUTRITION

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Interim Safety and Risk Assessment of Melamine and its Analogues in Food for Humans[a]

BACKGROUND

On September 11, 2008, FDA learned that melamine may be contained in an infant formula manufactured by a firm in China. As of September 21, 2008, FDA learned that a total of 52,857 cases of nephrolithiasis (and, in some instances, renal failure) had been reported in China linked to consumption of this contaminated powdered formula. There have been approximately 13,000 hospitalizations, and at least 3 deaths have been confirmed to date. The vast majority of illnesses involved children under the age of 3 years (82% < 2 years; 17% 2-3 years; 0.8% > 3 years; and no cases involved adults). The results of an investigation conducted in China indicated that Chinese-produced powdered infant formula was linked to these illnesses; no cases were associated with liquid infant formula. An investigation of powdered formulas was conducted nationally by China's General Administration of Quality Supervision, Inspection and Quarantine (AQSIQ) and revealed contamination of powdered formulas produced by 22 companies. Test results conducted in China on samples of the powdered infant formula showed that they contained a wide range of concentrations (0.1 ppm to greater than 2,500 ppm melamine). In addition, other countries have reported detection of melamine in other product categories, such as confections and beverages. We note that there are generally available analytical methods that can reliably detect a level of 1 ppm melamine in some food matrices.

Illnesses were reported in different regions of China, including Ganzu, Shaanxi, Ningxia, Jiangsu, Henan, Jiangxi, Hubei, Shandong, Anhui and Hunan. A press release from the Ganzu Health Bureau on September 11, 2008 stated that during the first half of 2008, one hospital in Ganzu treated 16 infants who had kidney stones. The 16 infants varied in age from 5 months to 11 months; some of the cases reportedly developed renal insufficiency. In a record review conducted for the period 2006 to the present, hospitals in Ganzu identified a total of 59 cases of kidney stones in infants, including 1 infant who died. The cases were located in 24 Ganzu counties and most of the cases were from rural areas. All of the cases occurred in 2008; none were identified in 2006 and 2007. Medical authorities in China indicated that there were 14 cases in which information was available regarding the composition of kidney stones. Twelve of these cases had stones reportedly composed of dihydrate uric acid and urine ammonium. Stones of this composition were reportedly visualized by ultrasound and CT scan, but not by routine x-ray, indicating that some cases may have not been diagnosed.

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Melamine, in its chainlike "polymerized" form, has been used for decades in manufacturing of dishes, plastic resins, flame-retardant fibers, components of paper and paperboard and industrial coatings. It has only very limited exposure in foods from these food contact substance uses. The estimated level of melamine in food resulting from all of these uses is less than 15 µg/kg (0.015 ppm). Additionally, trichloromelamine is approved for use as a sanitizing agent on food processing equipment and utensils, except for milk containers and equipment. Trichloromelamine readily decomposes to melamine during its use as a sanitizer. Only very low levels of melamine in food would be expected to result from this use. There is no approved melamine use in direct addition to human or animal food in the U.S., nor is it permitted to be used as a fertilizer in the U.S., as it is in some parts of the world.

TOXICOLOGICAL STUDY RESULTS

The observed toxic effects of melamine alone in animals in controlled studies occur only following high-dose exposures. All information thus far indicates that melamine appears to be metabolically inactive or inert (i.e., it does not readily undergo any type of metabolic change). This information supports a reasonable probability that all species eliminate the originally ingested substance, melamine or its analogues, and not a metabolite. Some species excrete melamine more slowly than other species. For example, fish excrete melamine more slowly than rodents. In addition, whether adverse effects are observed in some species and not others may vary depending on the level of exposure and which melamine analogues are present. Additionally, one of the bases for differential toxicity to these substances is species-specific rates of elimination.

Melamine and its analogues - cyanuric acid, ammelide and ammeline - are assumed to be of equal potency and are referred to collectively below as melamine-analogues. Since there is limited information about the toxicity or pathology of the analogues compared to melamine, it is deemed prudent to make an assumption that these analogues have equal effects. There is evidence available now that when these melamine analogues, especially cyanuric acid, are available in the kidney simultaneously they can combine to cause renal pathology (see discussion below). The actual extent of renal injury associated with different melamine analogues and the relative concentrations leading to toxicity is under experimental study now.

Preliminary work suggests that lattice crystals composed of melamine and cyanuric acid, and possibly other substances, form in the kidney. This has been shown to take place at various dose levels and is a threshold- and concentration-dependent phenomenon that would not be relevant to low levels of exposure to single melamine-type compounds. The combination of melamine and cyanuric acid has been linked to acute renal failure in cats and dogs. Crystals from cats that died from pet food containing melamine and cyanuric acid were comprised of melamine combined with cyanuric acid.[1, 2]

Melamine-cyanurate crystals have been shown to develop in mice, pigs, cats and fish kidneys when dosed with the combination of both melamine and cyanuric acid.[2-4] The crystals that form in the pigs and fish are identical to those seen in cats. The crystals are a lattice of six molecules -- three of melamine and three of cyanuric acid -- held together by hydrogen bonds.

In mammals, the toxicity of melamine alone is low, with a half-life of approximately three to four hours. Available publications report the most sensitive value for oral 50% lethal dose (LD_{50} is the amount that kills one-half of the tested animals) is 3,161 mg/kg bw/d in rats.[5, 6] The most recently reported no-observed-adverse-effect-levels (NOAELs) are 63 mg/kg bw/d (13 weeks, oral with feed, in rats); 240 mg/kg bw/d (28 days, oral with feed, in rats); 417 mg/kg bw/d (14 days, oral with feed, in rats); and 1,600 mg/kg bw/d (13 weeks, oral with feed, in mice).[6] In addition, the most sensitive calculated NOAELs for oral reproductive and developmental toxicity in rats are 400 mg/kg bw/d (maternal) and 1,060 mg/kg bw/d (fetal), respectively.[5, 6] The most commonly observed toxic effects in animal experiments where melamine was administered orally include: reduced food consumption, body weight loss, bladder stones, crystalluria, epithelial hyperplasia of urinary bladder, and lowered survival rate. However, no kidney failure or clinical symptoms of kidney failure were observed from these studies, or in a dog study.[7, 8]

Additional findings include:

- Only one oral long-term dog study has been reported. Apart from crystalluria (excretion of crystals in the urine) in that study, no other toxic effects were observed in dogs fed 1,200 mg/kg bw/d for one year.[5, 7] A short term oral study in cats dosed with melamine alone, cyanuric acid alone or both melamine and cyanuric acid in combination found no clinical pathology in cats treated with the compounds singly. Crystals formed in the kidneys of cats exposed to both compounds (64 mg/kg bw/d, total) composed of 32 mg/kg bw/d of each compound.[4] No studies with human subjects have been reported for melamine, but there are limited data on oral cyanuric acid exposure.[9]
- Histopathological reports on pets that consumed melamine-contaminated pet food in 2007 indicate that intratubular crystal obstruction is the mechanism of renal failure. There is accumulating experimental evidence that ingestion of food contaminated with melamine and cyanuric acid leads to crystal formation in the kidney and subsequent kidney failure. Crystals were found in the kidneys, urinary bladder and urine (crystals are microscopic and thus much smaller than stones). The mechanism of toxicity has been proposed to be similar to uric acid nephropathy in humans, where crystals obstruct renal tubules causing acute renal failure.[10, 11]
- High (4,500 ppm in the diet which is equivalent to 263 mg/kg bw/d) and continuous (2 years) dietary exposure to melamine in controlled studies is associated with an increase in the production of bladder stones and an increased incidence of urinary bladder tumors in male rats.[6,8] Only rats that had bladder stones developed tumors in these studies.[12]
- The NOAEL for stone formation of melamine toxicity is 63 mg/kg bw/d in a 13-week rat study.[6] This value is the lowest NOAEL (nontoxic dose) for melamine exposure noted in the published literature and is used with human exposure assessments below to provide an estimate of human safety/risk.
- In mammals, the toxicity of cyanuric acid (CYA) administered by itself is low. The acute lethal dose (LD_{50}) has been reported as 3,400 mg/kg bw. Kidney toxicity is

observed in mice at very high dose (80,000 mg/kg bw/d); rats show kidney toxicity at 5,400 mg/kg bw/d). The time taken to clear 50% of an administered dose: dogs - 2 hours; rats - ½ -1 hour[13].

SAFETY/RISK ASSESSMENT FOR INFANT FORMULA

FDA in collaboration with the Food Safety and Inspection Service (FSIS) of the Department of Agriculture, and in consultation with the Centers for Disease Control and Prevention (CDC, the Environmental Protection Agency (EPA) and the Department of Homeland Security (DHS) developed a TDI (Tolerable Daily Intake) for melamine and its analogues during the pet food contamination event of 2007.[b] It was based on results from animal studies with melamine and cyanuric acid administered alone. We designated 0.63 mg/kg bw/d as the tolerable daily intake, or TDI. The TDI is defined as the estimated maximum amount of an agent to which individuals in the population may be exposed daily over their lifetimes without appreciable health risk.[c]

This TDI was used to provide the basis for the safety/risk assessment in FDA's May 2007 Interim Melamine and Analogues Safety/Risk Assessment for food products from animals fed feed containing melamine compounds. In the present exposure event, the potential risk for toxicity from consumption of infant formula contaminated with melamine and its analogues is far higher than last year's risk of toxicity to humans from consumption of animals that had been inadvertently fed contaminated feed.

The previous assumptions that US FDA made in the 2007 risk/safety assessment regarding the pet food contamination episode cannot be applied to the current situation because the contaminated product represents the totality of caloric exposure for most of these infants; the exposure is chronic over months; the persons ingesting the products are infants and toddlers whose renal systems are not yet fully developed; and the exposure is not mitigated by previous passage through the digestive system of an animal. Moreover, several significant gaps in our scientific knowledge about melamine and its analogues toxicity regarding infants exist, including:

1. The impact of the presence of more than one melamine analogue which has the potential to increase the toxicity of the adulterated infant formula.
2. The consequences of continuous use of these infant formulas as sole source of nutrition.
3. The possibility that these formulations can be fed as the sole source of nutrition to premature infants with immature kidney function and even greater intake of infant formula per unit body weight for a longer time period than term infants.

Thus, the US FDA cannot establish a level of melamine and its analogues in these products that does not raise public health concerns.[d]

SAFETY/RISK ASSESSMENT FOR FOOD AND FOOD INGREDIENTS OTHER THAN INFANT FORMULA

The 2007 estimate of the TDI for melamine and its analogues serves as the starting point

for the present risk assessment.[e] That TDI is 0.63 mg/kg bw/d and is based on the results of a 13-week rat study. We describe below the procedure used to calculate a level of melamine and its analogues in food that does not raise public health concerns. A 100-fold safety factor is often accepted as an adequate margin between the lowest no-observed-adverse-effect level (NOAEL) from animal data and the TDI (tolerable daily intake) for humans, such that:

> 63 mg melamine and its analogues/kg-bw/d (NOAEL) divided by 100-fold safety factor=0.63 mg melamine and its analogues/kg-bw/d (TDI).

More recent studies[2,4] indicate that increased toxicity results from combined exposure to melamine and cyanuric acid. This raises a high degree of uncertainty with regard to the determination of safety/risk.[f] Given these conditions, the FDA has applied an additional 10-fold safety factor, yielding a combined safety factor of 1000-fold, to compensate for these uncertainties.

> 0.63 mg melamine and its analogues/kg-bw/d divided by added 10-fold safety factor = 0.063 mg melamine and its analogues /kg-bw/d.

The next step is to convert from a dose of 0.063 mg/kg bw/d to total melamine and its analogues consumed per day.

> 0.063 mg/kg-bw/d x 60 kg/person = 3.78 mg melamine and its analogues/person/day.

To estimate the level of melamine that does not raise public health concerns,

FDA used a worst case exposure scenario in which one-half of a person's total daily dietary intake (typically estimated at 3 kg (composed of liquid [1.5 kg] and solid food [1.5 kg]) is contaminated with melamine and its analogues. The previously determined (see above) total amount of melamine and its analogues/person/day:

> 3.78 mg melamine and its analogues/person/day divided by 1.5 kg of food = the food contamination level that would provide this amount of melamine and its analogues to a 60 kg person per day. Thus, 3.78 mg melamine and its analogues divided by 1.5 kg of food = 2.5 mg melamine and its analogues/kg food.

Therefore, if 50% of the diet were contaminated at a level of 2.5 ppm of melamine and its analogues, a person's daily intake would equal 0.063 mg/kg bw/d.[d]

The current incident has focused on melamine contamination of milk and milk-derived ingredients from China. As illustrated in Table 1, U.S. consumers would be exposed to only 1.1 % of the melamine TDI/10 (0.063 mg/kg bw/d) if all of the major milk-derived ingredients listed below were contaminated at a melamine level of 2.5 mg/kg (2.5 ppm) assuming an average per capita ingredient intake.

Table 1
U.S. Per Capita Melamine Intakes from Milk-derived Food Ingredients as a % of the TDI/10 if Melamine is Present at 2.5 mg/kg Concentration[1]

Ingredient	Per Capita Ingredient Intake[1] g/person/day	Per capita melamine intake (µg/p/d)[2]	Intake (% TDI/10)[3]
Casein	0.94	2.3	0.06
Lactose	2.2	5.5	0.15
Whey Protein Concentrate	1.7	4.3	0.11
Dry Whey	5	12.5	0.33
Dry Whole Milk	0.13	0.33	0.009
Nonfat Dry Milk	5.5	13.7	0.36
Skim Milk Powders	1.2	3.0	0.08
Dry Buttermilk	0.3	0.75	0.02
Total Exposure[4]	17	42.5	1.1

[1] Melamine assumed to be present at 2.5 mg/kg concentration.

[2] µg/p/d = micrograms/person/day

[3] Divide % TDI/10 by 100 to determine multiple of TDI/10. TDI is 0.63 mg/kg bw/d.

[4] Summation of per capita melamine intakes of all eight ingredients.

In summary, excluding infant formula and assuming that 50% of the diet is contaminated at a level of 2.5 ppm melamine and its analogs, there is a 1000-fold difference between the estimated dietary exposure (intake) and the level of melamine that does not cause toxicity in animals (NOAEL). Thus, levels of melamine and its analogues below 2.5 ppm in foods other than infant formula do not raise public health concerns.

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13. IUCLID Chemical Data Sheet: Substance ID 108-80-5, 65 pages, February 18, 2000.

[a] FDA issued its Interim Melamine and Analogues Safety/Risk Assessment on May 25, 2007. The May 2007 interim safety/risk assessment describes the risk to human health associated with eating pork, chicken, fish and eggs from animals that had been inadvertently fed animal feed adulterated with melamine and its analogues. The current document uses the same Tolerable Daily Intake (TDI) as the May 2007 interim assessment and considers additional scientific data now available.

[b] Interim Melamine and Analogues Safety/Risk Assessment, May 25, 2007. (http://www.cfsan.fda.gov/~dms/melamra.html).

[c] http://www.who.int/ipcs/methods/harmonization/areas/ipcsterminologyparts1and2.pdf

[d] We note that there are generally available analytical methods that can reliably detect a level of 1 ppm melamine in some food matrices.

[e] http://www.cfsan.fda.gov/~dms/melamra.html

[f] There are ongoing studies by the National Center for Toxicological Research (NCTR) and others designed to reduce the uncertainty with regard to determining the safety/risk of the combined presence of melamine and its analogues in food.

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