Interim Melamine and Analogues Safety/Risk Assessment

May 24, 2007

Executive Summary

This interim melamine and analogues 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 that may have been adulterated with melamine and its analogues (cyanuric acid, ammelide and ammeline). It was prepared by the Food and Drug Administration (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). We developed this safety/risk assessment in response to our ongoing investigation of contaminated vegetable protein products imported from China that were mislabeled as "wheat gluten" and "rice protein concentrate." Based on currently available data and information, the results of the safety/risk assessment indicate that the consumption of pork, chicken, domestic fish, and eggs from animals inadvertently fed animal feed contaminated with melamine and its analogues is very unlikely to pose a human health risk.

Chemical Identification Information for Melamine

Chemical Name:	Melamine
CAS Number:	108-78-1
Molecular Weight:	126.12
Molecular Formula:	$C_3H_6N_6$
Melting Point:	<250^0 C.

Chemical Synonyms: 02284 (CA DPR Chem Code), 1,3,5-Triazine-2,4,6-triamine, 108-78-1 (CAS Number), 108781 (CAS Number), 2,4,6-triamino-1,3,5-triazine, 2,4,6-triamino-s-triazine, 2284 (CA DPR Chem Code), 777201 (US EPA PC Code), Cyanuramide, Cymel, Cyromazine breakdown product, Melamine, S-triaminotriazine, Triaminotriazine

Additional chemical information for melamine and analogs is available in Appendix I.

Toxicological Profile

As discussed in more detail in this section, the observed toxic effects of melamine on animals in controlled studies only occur following high dose exposures. Differential toxicity is seen in different animal models. It is believed that this differential toxicity is due to species-specific toxicokinetics. 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 monogastric species eliminate the originally ingested substance, melamine, or its analogues, and not a metabolite. In addition, whether adverse effects are observed in some species and not others may vary depending on the level of exposure.

Melamine and melamine analogues (cyanuric acid, ammelide and ammeline) are assumed to be of equal potency and will be referred to collectively in this assessment as melamine compounds (MC). While it is entirely possible that the analogues are more or less potent than the parent compound, melamine, we have no information that assesses the relative potency of the three analogues as compared to melamine; therefore, for the purpose of this interim assessment, we have made an assumption of equal potency. It has been hypothesized that melamine may interact synergistically with its three analogues, but no studies have been conducted that specifically test this hypothesis. Very preliminary work suggests that if it does occur, the formation of lattice crystals, particularly between melamine and cyanuric acid, takes place at very high dose levels and is a threshold and concentration dependent phenomenon that would not be relevant to low levels of exposure. Although still under investigation, it now appears that the combination of melamine and cyanuric acid has been linked to the acute renal failure in cats and dogs that have eaten the suspect pet foods (http://www.labservices.uoguelph.ca/urgent.cfm). The toxicity of melamine to mammals is low. Available publications report the most sensitive value for oral 50% lethal dose (LD_{50}) is 3161 mg/kg in rats.[1, 2] The most recent reported no-observed-adverse-effect-levels (NOAELs) are 63 mg/kg bw/day (13 weeks, oral with feed, in rats); 240 mg/kg bw/day (28 days, oral with feed, in rats); 417 mg/kg bw/day (14 days, oral with feed, in rats); and 1600 mg/kg bw/day (13 weeks, oral with feed, in mice).[2] In addition, the most sensitive calculated NOAELs for oral reproductive and developmental toxicity in rats are 400 mg/kg bw/day (maternal) and 1060 mg/kg bw/day (fetal), respectively.[1, 2] The most commonly observed 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, including in a dog study.[3, 4]

Studies of melamine have been conducted in rodents.[1, 2] Only one oral long-term dog study has been reported. Apart from crystalluria (excretion of crystals in the urine) in that study, no toxic effects were observed in dogs fed 1200 mg/kg/day for one year.[1, 3] No oral studies with cat or human subjects have been reported.

Brief histopathological reports from affected pets indicate that acute/subchronic kidney failure is the mechanism of toxicity. It is still unclear whether there is a direct relationship between the kidney failure and crystallization in the kidneys subsequent to purported melamine consumption, although crystals were found in the kidneys and urinary bladder (crystals are much smaller than stones and their presence does not necessarily result in tissue/organ pathology or dysfunction).
High (4500 ppm or 263 mg/kg bw/day) 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.[2, 4] The two incidences are highly associated with each other.[5]

The NOAEL for stone formation of melamine toxicity is 63 mg/kg bw/day in a 13-week rat study.[2] This value is the lowest NOAEL noted in the published literature and is used with human exposure assessments below to provide an estimate of human safety/ risk.

Melamine and Analogues Intake Scenarios

The exposure scenarios presented below pertain to melamine compounds. As stated previously, we have assumed that all MCs are equipotent and we are treating them as a group of compounds and not as single compounds. Scenario 1 presents intake estimates for pork, chicken, egg and fish consumption. Scenario 2 presents intake estimates for foods that contain meat, poultry and pork. Scenario 3 is an intake estimate that is based on, as a worst case assumption, that melamine is in all solid food. For all three scenarios, intake estimates were based on food consumption levels for a 60-kg individual (the norm for exposure analysis by FDA/CFSAN's Office of Food Additive Safety).

Accumulation of melamine in the edible tissue of animals (pork, poultry and fish) consuming the contaminated feed at the levels reported in feed to date is considered highly unlikely. For the purpose of this assessment it is assumed that all edible tissues contain 50 ppb melamine. The basis for using such a level comes from reports from FDA and FSIS of trace levels of melamine in tissues from some hogs that consumed the suspect feed containing rice protein. The 50 ppb level represents a conservative estimate of the Limit of Detection (LOD) for the methods employed; a value that will ensure that the exposure scenarios are in turn conservative. (The LOD is the level below which a contaminant cannot be measured with current analytical methods.) Currently this method is designed to only detect melamine in meat; however, it is presumed that the other MCs are present in the tissue. For the purposes of this assessment, the concentration of melamine assumed in the tissue (50 ppb) is doubled to 100 ppb to account for cyanuric acid, which was not actually measured by the test. Doubling the measurable tissue melamine level was based in part on the relative levels of MC compounds (analogues levels were generally no greater than melamine levels) observed in the contaminated feed and in part on the identification of the crystals in the kidneys being a mixture of melamine and cyanuric acid. This suggests that cyanuric acid might be a greater concern than the other analogues and doubling should more than account for cyanuric acid based on currently available data. Therefore, in lieu of any reported residue values for MCs in edible tissues and based on the reported levels of MCs in the suspect feeds and the short half life and rapid excretion of MCs, the use of 100 ppb as a default residue level was considered reasonable for the purpose of determining safety. Inherent in this value is another conservative assumption that all meat/poultry/fish/egg tissue was from animals that had been fed contaminated feed until just prior to slaughter or collection. Due to the rapid excretion of melamine and cyanuric acid and because these compounds are not metabolized, withdrawing contaminated feed would be expected to lead to a relatively rapid clearance of the compounds from the animal.

FSIS has recently completed a "Swine and Poultry Exposure Assessment," attached as Appendix II. FDA has recently completed sampling of two commercial fish farming operations; the report of this sampling is attached as Appendix III. These data and information confirm and re-enforce the assumptions and conclusions of this interim safety/risk assessment.

Scenario 1
(Direct intake of catfish, chicken, eggs, and pork)

Intake estimates of MCs from catfish, chicken (chicken parts only; we did not consider chicken as an ingredient in other dishes), pork (chops, steaks, cutlets, ham, and roast), eggs ("breakfast-type" egg consumption only) for the mean and 90th percentiles are presented in Table 1. We assumed 100 ppb MC contamination for all foods included in the intake estimates.

All intake estimates are for the U.S. population 2 years of age and above using food intake data from the USDA Continuing Survey of Food Intake by Individuals (CSFII, 1994-1996 and 1998 Supplemental Children's Survey).

Only one user in the survey population consumed kidneys. As a result kidney consumption was not included in the intake estimates.

Melamine intake among individuals who consume listed food types, assuming 100 ppb MC contamination, 2-day average

Food Type	**% eaters**	**Mean**	**90th %**
Catfish	1%	10 µg/p/d (0.16 µg/kg-bw/d)	20 µg/p/d (0.34 µg/kg-bw/d)
Chicken (breast, leg, drum, thigh, wing)	29%	6.4 µg/p/d (0.11 µg/kg-bw/d)	12 µg/p/d (0.21 µg/kg-bw/d)
Eggs	25%	4.5 µg/p/d (0.081 µg/kg-bw/d)	8.4 µg/p/d (0.16 µg/kg-bw/d)
Pork (chops, steaks, cutlets, ham, roasts)	14%	5.1 µg/p/d (0.085 µg/kg-bw/d)	9.8 µg/p/d (0.17 µg/kg-bw/d)
Combined Catfish, Chicken, Eggs and Pork	54%	7 µg/p/d (0.12 µg/kg-bw/d)	14 µg/p/d (0.24 µg/kg-bw/d)

Scenario 2
(Intake of products made with chicken and pork meat and byproducts)

Intake was calculated using food consumption data from the 1994 – 1996, 1998 CSFII and the commodity codes for chicken, pork, and both chicken and pork meat and byproducts combined. Commodity codes were used to represent a comprehensive scenario for intake of MCs from contaminated chicken, pork, and chicken and pork. Commodity codes take into account the intake of breast meat, cutlets, etc., as well as the amount of chicken or pork in a particular food, by drawing on data for recipes that are available in the database (i.e., the amount of pork in a hot dog or the amount of chicken in lunch meat). The table below indicates the eaters-only intake of melamine (assuming a contamination level of 100 ppb MCs in the meat) from the consumption of chicken, pork, or both chicken and pork contaminated with MCs.

Table 2: Melamine intake among individuals who consume listed food types, assuming 100 ppb MC contamination, 2-day average

Commodity Type	% eaters	Mean	90th %
Chicken and Pork, meat and byproducts	92%	5 μg/p/d (0.08 μg/kg-bw/d)	10 μg/p/d (0.18 μg/kg-bw/d)
Chicken, meat and byproducts	63%	4 μg/p/d (0.07 μg/kg-bw/d)	9 μg/p/d (0.16 μg/kg-bw/d)
Pork, meat and byproducts	78%	2 μg/p/d (0.04 μg/kg-bw/d)	6 μg/p/d (0.10 μg/kg-bw/d)

Scenario 3
(Worst case)

Assumptions:

- MCs is present in all solid food at a level of 100 ppb (μg/kg)
- A person consumes 3000 g (or 3 kg) of food per day, of which 1500 g is from solid food

MC Intake from solid food = (1.5 kg food/p/d) x (100 μg MCs/kg food)
= 150 μg MCs per person per day or 2.5 μg/ kg bw/day for a 60 kg individual

Margins of Safety and Levels of Concern for Melamine Compounds

The point of departure (POD) is the NOAEL of 63 mg/kg/day from the rodent subchronic bioassay. This POD was then divided by two 10-fold safety/uncertainty factors (SF/UF) to account for inter- and intra-species sensitivity, for a total SF/UF of 100. The resulting Tolerable Daily Intake (TDI) is 0.63 mg/kg bw/day. The TDI is defined as the estimated maximum amount of an agent to which individuals in a population may be exposed daily over their lifetimes without an appreciable health risk with respect to the endpoint from which the NOAEL is calculated.[6]

In considering all of the exposure estimates presented above, including the 90th percentile estimates from Scenario 1 (Table 3), all are well below the TDI of 0.63 mg/kg/day or 630 μg/kg bw/day and have large Margins of Safety (MOS). The MOS is defined as the TDI divided by the estimate of exposure. For Scenario 2, the MOS would be approximately double that for Scenario 1. Indeed the highest exposure estimate for the worst case scenario (Scenario 3) of 2.5 μg/kg bw/day is 252 times lower than the TDI.

Table 3: Margins of Safety (MOS) for Scenario 1

Food Type	90^{th} percentile intake (µg/kg bw/day)	MOS
Catfish	0.34 µg/kg-bw/d	1853
Chicken (breast, leg, drum, thigh, wing)	0.21 µg/kg-bw/d	3000
Eggs	0.16 µg/kg-bw/d	3938
Pork (chops, steaks, cutlets, ham, roasts)	0.17 µg/kg-bw/d	3706
Combined Catfish, Chicken, Eggs and Pork	0.24 µg/kg-bw/d	2625

When considering what concentrations of MCs might be of concern in food, it is important to take into account the amount of a given food product that is eaten by an individual per day. Table 4 shows the "Level of Concern" (LOC) calculated for pork, poultry, eggs and catfish. The LOC takes into account the consumption of each product (based on CSFII data) and the TDI of melamine. These LOCs represent levels of MCs in the products below which there is no appreciable risk. The following table contains LOCs for animal-derived foods. Each value is derived on the basis of a consumption amount and the TDI according to the following formula where the body weight used to derive these values is 60 kg:

$$\text{LOC} = \text{TDI (0.63 mg/kg/day) x 60 kg/ } 90^{th} \text{ percentile consumption level}$$

Table 4: Levels of Concern (LOC) for Total MCs

Food	Consumption* 90^{th} level g/person/day	LOC µg/g
Pork	98	386
Poultry	117	323
Eggs	84	450
Catfish	195	194

* Food consumption levels used for the food types from Table 1 used to derive LOCs.

In light of the calculation that the highest estimate of exposure (i.e., 2.5 µg/ kg bw/day for a 60 kg individual) is more than two orders of magnitude lower than the TDI (0.63 mg.kg.day), which are based on reasonable assumptions and a 100-fold safety factor, the consumption of pork, poultry, eggs and domestic fish is very unlikely to pose a human health risk.

Data Needs and Research Recommendations

Information on toxicity of MC and on the levels of MC in edible tissues would be useful for future assessments. This research could include:

- Analytical method improvement to confirm low levels in tissues, including development and dissemination of standards;
- Characterization of the crystals found in experimental animals and clinical cases;
- Basic toxicological studies in several species, especially comparative renal effects;
- Further examination of the relative toxicity of melamine and analogues and the toxicity of co-exposures and the determination of the role of additivity and synergism in MC toxicity; and
- Development of biomarkers for onset of MC-type renal failure for better clinical diagnoses.

References

1. IUCLID Chemical Data Sheet: Substance ID 108-78-1, p. 30-98, February 18, 2000
2. OECD SIDS Analysis UNEP Publications: Melamine, June, 2002
3. Clayton, G. D. and FE Clayton (eds.) Patty's Industrial Hygiene and Toxicology. Vol. 2A, 2B, 2C: Toxicology. 3rd Ed. New York: John Wiley Sons, 1981-1982., p.2771
4. U.S. Department of Health and Human Services: NTP Technical Report, TR 245, 1983
5. Memorandum by CFSAN Cancer Assessment Committee dated March 17, 1983
6. http://www.who.int/ipcs/methods/harmonization/areas/ipcsterminologyparts1and2.pdf

SUPPORTING DATA AND INFORMATION
(3 Appendices)

APPENDIX I

Chemical and Physical Characteristics of Melamine and Its Analogs

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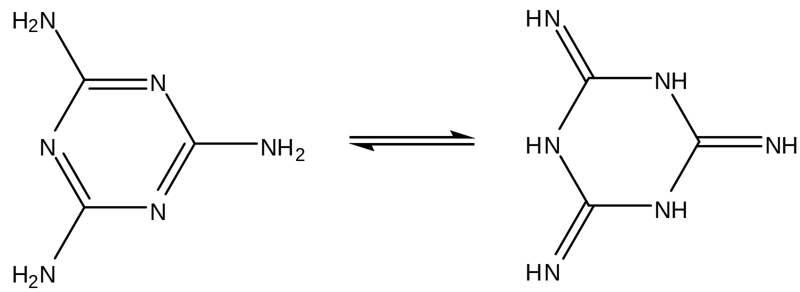
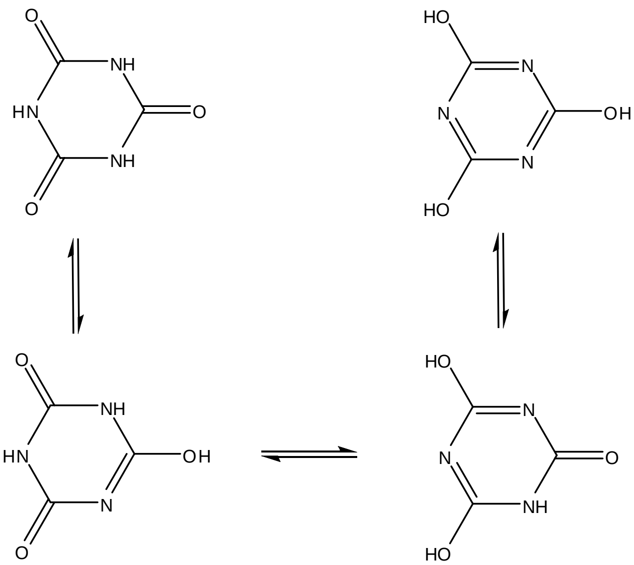
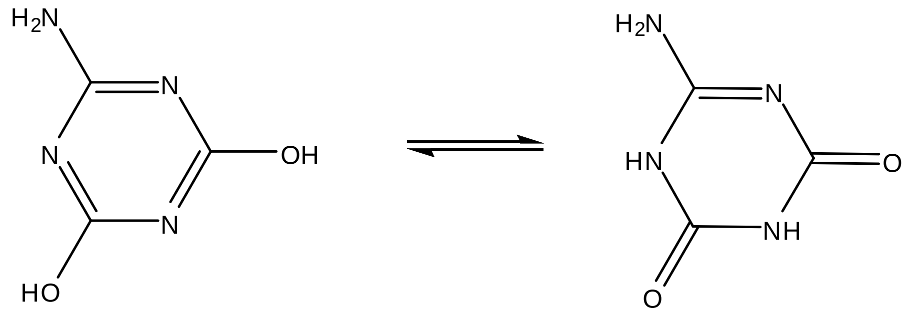
MELAMINE

Synonym(s)	Melamine, 8CI 2,4,6-Triamino-*s*-triazine Cyanuramide Cyanurotriamide 2,4,6-Triamino-1,3,5-triazine Virset 656-4
Structure	
Chapman & Hall Number	JKW82
CAS Registry Number	108-78-1
Type of Compound Code	PM7260 PA3150 PS8300 PS8120
Molecular Formula	$C_3H_6N_6$
Molecular Weight	126.121
Accurate Mass	126.065394
Percentage Composition	C 28.57%; H 4.79%; N 66.63%
General Statement	The triimino tautomer is known only as derivs. Intermediate tautomers also theoretically possible
Source / Synthesis	Manuf. from dicyandiamide or urea
Use / Importance	Used in synthesis of melamine resins. Used as a 0.2% aq. soln. for photometric detn. of Pd. Reference material used in elemental microanalysis
Physical Description	Cryst.
Melting Point	Mp 347° dec.
Solubility	Spar. sol. H_2O; sl. sol. EtOH; insol. Et_2O, C_6H_6, CCl_4
Dissociation Constant	pK_a 5.35 (25°, H_2O)
Other Data	Subl. when gently heated. Hydrol. rapidly by water
Hazard and Toxicity	LD_{50} (rat, orl) 3161 mg/kg. Exp. carcinogen
RTECS Accession Number	OS0700000

Cyanuric acid

Synonym(s)	 1,3,5-Triazine-2,4,6(1*H*,3*H*,5*H*)-trione, 9CI *s*-Triazine-2,4,6-triol, 8CI Isocyanuric acid Trihydroxycyanidine
Structure	
Chapman & Hall Number	JZQ62
CAS Registry Number	108-80-5
Molecular Formula	$C_3H_3N_3O_3$
Molecular Weight	129.075
Accurate Mass	129.017442
Percentage Composition	C 27.92%; H 2.34%; N 32.55%; O 37.19%
General Statement	Tri-*NH*-form predominates. Derivs. of the tri-*OH* tautomer are referred to as cyanurates and of the tri-*NH* form as isocyanurates
Use / Importance	Commercially available. Used in rubber, resin manuf.
Biological Use / Importance	Herbicide. Ethers (cyanuric esters) are hypnotics and anticonvulsants
Physical Description	Off-white cryst. + $2H_2O$ (H_2O)
Melting Point	Mp 360°
Solubility	Sol. hot EtOH, mod. sol. H_2O
Density	d^0 1.77
Dissociation Constant	pK_a 4.74 (25°, H_2O)
Other Data	Dec. to cyanic acid on heating without melting. Reacts as mono-, di- or tribasic acid
Hazard and Toxicity	Reacts violently with EtOH. Forms explosive by-product with chlorine. Eye and skin irritant. LD_{50} (rat, orl) 7700 mg/kg
RTECS Accession Number	XZ1800000

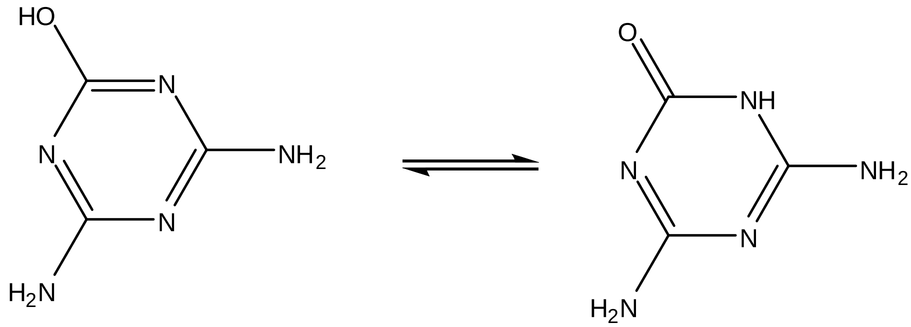
AMMELIDE

Synonym(s)	Ammelide Melanuric acid Cyanuric acid monoamide 6-Amino-1,3,5-triazine-2,4(1*H*,3*H*)-dione, 9CI
Structure	
Chapman & Hall Number	GBG07
CAS Registry Number	645-93-2
Molecular Formula	$C_3H_4N_4O_2$
Molecular Weight	128.09
Accurate Mass	128.033426
Percentage Composition	C 28.13%; H 3.15%; N 43.74%; O 24.98%
General Statement	Dioxo form predominates. Other tautomers also possible
Physical Description	Cryst. (H_2O)
Other Data	Dec. at high temp. without melting

References

Roginskaya, T.N., *et al., Zh. Fiz. Khim*, 1971, **45**, 1609, *(tautom)*
Yamomoto, H., *et al., Chem. Ber*, 1973, **106**, 3194, *(synth)*
Moschel, R.C., *et al*., *J.O.C.*, 1974, **39**, 1983, *(synth)*
Matsui, M., *et al*., *J.O.C* , 199 , **56**, 498, *(synth, pmr, ms)*

AMMELINE

Synonym(s)	4,6-Diamino-1,3,5-triazin-2(1*H*)-one, 9CI 4,6-Diamino-*s*-triazin-2-ol, 8CI Cyanuric acid diamide Ammeline
Structure	
Chapman & Hall Number	GBG08
CAS Registry Number	645-92-1
Molecular Formula	$C_3H_5N_5O$
Molecular Weight	127.105
Accurate Mass	127.049410
Percentage Composition	C 28.35%; H 3.96%; N 55.10%; O 12.59%
General Statement	*OH*-Tautomer more stable and predicted to predominate in gas phase. There is little evidence to support the general supposition that the *NH*-tautomer is generally favoured (see Wang et. al.)
Use / Importance	Used in lubricating greases etc., much patented
Physical Description	Cryst. (Na_2CO_3 aq.)
Other Data	Salts unstable in H_2O

APPENDIX II
Swine and Poultry Exposure Assessment
May 24, 2007

Investigations of contamination of pork and poultry with melamine compounds (melamine, cyanuric acid, ammeline and ammelide; MCs) by FSIS and FDA have extended into a number of states. Samples have been collected from pet food manufacturers, feed mills, warehouses, and farms. Samples include animal feed components (pet food scrap and bakery feeds that contain pet food scraps), hog and poultry animal feed, tissue and urine samples from swine, and tissue samples from poultry. This appendix includes a summary of the laboratory and inspection activity results through May 18, 2007. These data support the assumptions made in the human health risk assessment and, together with the human health risk assessment, provide the basis for FSIS' decisions to release animals and not to recall products.

Investigations have determined that pet food scraps contaminated with MCs in protein concentrates from China were added to animal feed. This FDA investigation indicates that protein concentrates comprise 5-6% of the pet food scraps. It is important to understand feed production and the percentage of pet food scraps in the various feeds. This provides information on the potential range of swine and poultry exposures. Those animals with the highest exposures would be expected to have the highest tissue concentrations. In this investigation it was determined that for swine, pet food scraps typically are mixed with grain to make hog feed, with the scraps comprising 5-10% of the final feed. Therefore, the concentration of MCs in the hog feed typically would be much lower than that found in the pet food. Investigations have also shown, however, that some hogs were fed 50-100% pet food scraps. Those hogs would be expected to have the highest exposures and subsequent tissue concentrations of melamine. It was also determined in this investigation that for poultry, the pet food scraps are first mixed with bakery scraps to form bakery meal. That bakery meal is then mixed with other feed components to make chicken feed. Information indicates that bakery meal could comprise from 3-15% of the chicken feed. This additional step with the bakery meal further dilutes the MC concentrations in poultry feed as compared to that in hog feed.

Concentrations of Melamine and Related Compounds in Feed

Pet Food Scrap Samples

Approximately 57 samples of pet food scraps destined for swine or poultry feed were collected from 14 locations thought to have received contaminated product. The results of the analyses are presented in Table A-1. Of those samples analyzed to date, 30 tested negative for melamine. The range for other pet food samples, when quantitative amounts were reported, was 9.4 to 1,952 ppm melamine. Results for cyanuric acid were reported for 20 samples. Of those, 13 were negative and the highest value quantitated was 2,180 ppm. Information was available on ammelide and ammeline in 20 samples; 18 samples were negative and two were positive for both compounds. For ammelide, the two concentrations that were positive were 6.0 and 10.8 ppm, and for ammeline were 3.0 and 43.3 ppm.

Bakery Meal Samples

As can be seen in Table A-2, to date, 27 samples of bakery meal thought to have been made with contaminated pet food have been collected and analyzed for all four compounds from four locations. Of those, 24 were negative for melamine. The three positive samples were from two different locations and contained concentrations of melamine from 10.6 to 59.6 ppm. Cyanuric acid was not detected in 15 of the samples, and ranged from 1.8 to 146.3 ppm in the other 12 samples. Nine samples had detectable levels of ammeline (Range of 1.2 to 24.9 ppm) and the other 18 samples were negative. Ammelide was detected in one sample (20.1 ppm) and the other 26 samples were negative.

Swine Feed Samples

A total of 17 swine feed samples have been collected from seven farms or feed mills (Table A-3). Of those, ten were negative for melamine, and in those quantitated, concentrations for melamine ranged from 30 to 120 ppm. Information on melamine analogues (cyanuric acid, ammeline, and ammelide) in swine feed is available in six samples. Cyanuric acid was present in three samples (range, 6.6 to 22.52 ppm) and negative in three samples. Ammeline was present in two samples (5.6 and 10.8 ppm) and negative in four samples. Ammelide was present in two samples (33.6 and 43.2 ppm) and negative in four samples.

Poultry Feed Samples

Results are available for 21 poultry feed samples for the four analogues from two locations (Table A-3). All 21 samples were negative for melamine. Two samples were positive for cyanuric acid (2.11 and 2.63 ppm), one was positive for ammelide (13.9 ppm), and all other results were negative.

Concentrations of Melamine in Animal Tissues

As discussed in the human health risk assessment, for the purpose of this assessment it is assumed that all edible tissues contain 50 ppb melamine. The basis for using this level comes from the concentration of melamine measured in hog tissues. The method employed can adequately detect levels of melamine of 50 ppb and greater in hog tissues, and while the method may be able to detect lower levels, the 50 ppb values is used in the current document (and the safety/risk assessment) as a conservative estimate of the Limit of Detection (LOD). The concentrations of melamine measured in hog tissues are presented in Table A-4. The melamine concentrations measured in meat from four of the hogs exposed to the highest percentage of contaminated pet food scraps (Location B) were below 50 ppb. Meat samples from seven hogs from three different locations were also below the 50 ppb level. It should be noted that the 50 ppb that represents a conservative estimate of the ability of the method used to detect melamine will ensure that the exposure scenarios are in turn conservative.

In addition to measuring melamine concentration in hog meat, kidney tissues have also been analyzed. Concentrations of melamine in the kidneys of the hogs from Location B were below 50 ppb, and histological examination of the tissue did not show evidence of crystal formation. Crystal formation was also not seen in exposed hogs from Location A. FSIS conducts gross examinations on the kidneys of hogs at slaughter; if there is inflammation or enlargement of the kidney, the condition will be classified as nephritis in FSIS' electronic Animal Disposition Reporting System (eADRS). In eADRS, between January 1 and May 9, 2007, out of almost 5 million hogs slaughtered at the facilities known to have received hogs fed contaminated feed, only 235 hogs have been suspected of having nephritis, and only 15 hogs have been condemned because of nephritis. Those rates are less than observed during the same time period in all other establishments (see Table A-5).

Subsequent to the development of the method for hog tissue, a method has been validated for poultry tissue at the 50 ppb. The melamine concentrations measured in poultry tissue samples from six birds from two locations exposed to contaminated feed were all below the 50 ppb level. This supports the use of 50 ppb as the melamine concentration in the scenarios below. The kidneys from those birds were also examined; no significant lesions and no crystals were seen.

Excretion of Melamine Over Time

The University of California at Davis examined changes in melamine concentrations in hog urine and tissue samples from Location A (see Table A-6). (Note: the University of California at Davis validates the LOD at 10 ppb in hog tissue and 500 ppb in hog urine.) The hogs were likely fed melamine-contaminated feed for 14 days (from 4/4/07 through 4/18/07). Scrap pet food was the source of the contamination. The scrap pet food samples (2 tested) contained 69 and 74 ppm melamine and the scrap constituted 5-10% of the daily feed ration (a calculated end concentration in feed of 3.5 to 7 ppm). Samples of hog urine and tissue were collected over time. The first urine samples were collected on the day it was discovered that the feed was potentially contaminated with melamine; the hogs were not fed contaminated feed after that date (4/18/07). Urine samples were collected daily after that point and have been analyzed out to 14 days; tissue samples (ham and loin) have been collected and analyzed one day, seven days and fourteen days after contaminated feed use was halted.

Seven urine samples were taken from hogs without a period of withdrawal from contaminated feed; these hogs would be expected to have the highest melamine concentrations. The seven urine samples ranged from <500 to 940 ppb melamine. Urine and tissue samples were collected from three hogs one day after withdrawal from contaminated feed. Urine melamine concentrations were higher at that time and ranged from 1,020 to 2,220 ppb, and concentrations in ham and loin ranged from 9 to 12 ppb (based on the limit of detection reported by University of California at Davis). Pathology was conducted on these three hogs. No significant pathological findings were noted, including in kidney and urinary bladder tissues, and no birefringent crystals were detected with polarized light (birefringent crystals were seen in cats and dogs).

Urine and tissue samples were collected from two hogs 7 days after withdrawal from contaminated feed. All samples (urine, ham and loin) from one of the hogs were negative. Levels of melamine were present in the samples from the other hog, but could not be accurately quantified because it was below the LOD (urine ≤ 500 ppb; ham and loin ≤ 10 ppb). Six urine samples were analyzed daily on Day 8 through Day 14 post-withdrawal. One of the 6 samples from Day 9 had detectable levels of melamine (<500 ppb); all other of the 41 samples did not contain detectable levels of melamine.

In addition to the data on hogs from Location A and B, urine samples were collected from hogs at four other farms (Table A-6). Quantitative data was available for three farms. Data from two of those farms were within the same order of magnitude as those seen in Location A. Urine samples from the other farms were one order of magnitude higher than those seen in Location A. The reasons for the higher levels are unknown and no tissue samples are available from that farm.

Summary

As can be seen in Table A-4, no hog or chicken tissue samples have been measured to date that exceed the conservative validation level of the melamine assay of 50 ppb. As stated previously, this is true for even those hogs believed to have been feed the highest percentage of pet food scraps contaminated with melamine (Location B). Based on the urine assay as validated by University of California at Davis, melamine concentrations appeared to decrease in hogs withdrawn from contaminated feed for seven days. Such a decrease provides a further margin of safety for animals removed from potentially contaminated feed for a period of time prior to going to slaughter.

Table A-1: Analytical Results for Melamine Compounds in Pet Food Scraps*

Source Number	Number of Samples Collected	Melamine	Cyanuric Acid	Ammelide	Ammeline
1	4	Negative 9.4 ppm Negative Negative	352.3 2180 Negative Negative	Negative Negative Negative Negative	Negative Negative Negative Negative
2	7	Positive Positive Positive Positive Positive Positive Positive	NA NA NA NA NA NA NA	NA NA NA NA NA NA NA	NA NA NA NA NA NA NA
3	15	Negative Negative Positive Positive Positive Positive Negative Negative Negative Negative Negative 83.2 Negative Negative Negative	NA NA NA NA NA NA NA NA NA NA NA 22.5 15.0 6.6 Negative	NA NA NA NA NA NA NA NA NA NA NA 10.8 6.0 Negative Negative	NA NA NA NA NA NA NA NA NA NA NA 43.3 3.0 Negative Negative
4[a]	3	1,952 594 567	NS NS NS	NS NS NS	NS NS NS
5[a]	2	Positive Positive	NS NS	NS NS	NS NS
6	6	Negative Negative Negative Negative Negative Negative	NA NA NA NA NA NA	NA NA NA NA NA NA	NA NA NA NA NA NA
7	4	Positive Positive Negative Negative	NS NS NS NS	NS NS NS NS	NS NS NS NS
8	1	Positive	NA	NA	NA
9	1	Negative	Negative	Negative	Negative
10	1	Negative	Negative	Negative	Negative
11	6	Negative 17.5 17.5 Negative Negative 22	Negative Negative Negative Negative Negative 549	Negative Negative Negative Negative Negative Negative	Negative Negative Negative Negative Negative Negative

Source Number	Number of Samples Collected	Melamine	Cyanuric Acid	Ammelide	Ammeline
12	3	Negative Negative Negative	416 Negative Negative	Negative Negative Negative	Negative Negative Negative
13	1	Negative	Negative	Negative	Negative
14[b]	2	74 69	NA NA	NA NA	NA NA

Abbreviations: NA, Not Analyzed; NS, Not Specified.
[a] Analysis conducted at a private laboratory.
[b] Analysis conducted by UC Davis.

*Data are presented as concentrations in part per million (ppm) or qualitatively as positive or negative, depending on results reported. Analyses were conducted at FDA or private laboratories. Identifying information on location has been omitted. Results are reported as "Not Specified" if it is not clear whether the sample was tested for a given analogue.

Table A-2: Analytical Results for Melamine Compounds in Bakery Meal Samples*

Source Number	Number of Samples Collected	Melamine	Cyanuric Acid	Ammelide	Ammeline
1	5	Negative 19.28 Negative Negative Negative	3.0 6.6 1.8 Negative Negative	1.2 8.4 1.2 Negative Negative	Negative Negative Negative Negative Negative
2	6	Negative 10.6 59.6 Negative Negative Negative	Negative 4.1 23.9 Negative Negative Negative	Negative 3.8 24.9 Negative Negative Negative	Negative Negative 20.1 Negative Negative Negative
3	10	Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative	61.4 59.6 50.2 28.9 19.62 21.1 Negative Negative Negative Negative	Negative 3.3 2.67 1.88 Negative 1.36 Negative Negative Negative Negative	Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative
4	6	Negative Negative Negative Negative Negative Negative	146.3 Negative Negative Negative Negative Negative	Negative Negative Negative Negative Negative Negative	Negative Negative Negative Negative Negative Negative

*Data are presented as concentrations in part per million (ppm) or qualitatively as positive or negative, depending on results reported. Analyses were conducted at FDA or private laboratories.

Table A-3: Analytical Results for Melamine Compounds in Swine and Poultry Feed*

Source Number	Number of Samples Collected	Melamine	Cyanuric Acid	Ammelide	Ammeline
Swine Feed					
1	1	Negative	NA	NA	NA
2	6	30 50 60 120 60 Negative	NA NA NA NA NA NA	NA NA NA NA NA NA	NA NA NA NA NA NA
3	3	83.4 Negative Negative	22.52 15 6.6	10.8 5.6 Negative	43.2 33.6 Negative
4	2	Negative Negative	NS NS	NS NS	NS NS
5	1	Negative	NS	NS	NS
6	1	Positive	NS	NS	NS
7	3	Negative Negative Negative	Negative Negative Negative	Negative Negative Negative	Negative Negative Negative
Poultry Feed					
8	14	Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative	Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative	Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative	Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative Negative
9	7	Negative Negative Negative Negative Negative Negative Negative	Negative 2.11 2.63 Negative Negative Negative Negative	13.9 Negative Negative Negative Negative Negative Negative	Negative Negative Negative Negative Negative Negative Negative

Abbreviations: NA, Not Analyzed; NS, Not Specified.

*Data are presented as concentrations in part per million (ppm) or qualitatively as positive or negative, depending on results reported. Analyses were conducted at FDA or private laboratories. Identifying information on location has been omitted. Results are reported as "Not Specified (NS)" if it is not clear whether the sample was tested for a given analogue.

. **Table A-4: Analytical Results for Melamine in Swine and Poultry Tissue***

Location	Number of Samples Collected	Concentration Melamine
Swine		
Location A	Loin and ham samples from 3 hogs one day post exposure	< LOD (50 ppb) (Range 9 – 12 ppb) [a]
	Loin and ham samples from 2 hogs 7 days post exposure	Not detected in one hog; less than 10 ppb in other [b]
	Loin and ham samples from 2 hogs 14 days post exposure	Negative [b]
Location B	Meat and kidneys from 4 hogs	< LOD (50 ppb for meat and kidney)
Location C	2 meat samples [c]	< LOD (50 ppb)
Location D	Tissue from 2 hogs	< LOD (50 ppb)
Poultry		
Location E	Meat from 4 Chickens	< LOD (50 ppb)
Location F	Meat from 2 Chickens	< LOD (50 ppb)

Abbreviations: Level of Detection, LOD.
[a] Initial analysis conducted by University of California at Davis. FSIS Western laboratory repeated analysis and reported at the Level of Detection for the Western Laboratory of 50 ppb.
[b] Analysis conducted by University of California at Davis.
[c] Analysis conducted at a private laboratory.

* Data are presented as concentrations in part per billion (ppb) or qualitatively as positive or negative, depending on results reported. If a laboratory reported a concentration below the level of quantitation for the method, than the level of quantitation is presented followed by the reported value in parenthesis. It is important to note, however, that melamine can not be reliably measured below the level of quantitation, so conclusions that require that level of accuracy can not be made based on these data.

Table A-5: Nephritis Seen in Swine at Slaughter from January 1 through May 9, 2007. [a]

	Total Number of Hogs Slaughtered	**Total Number Nephritis Cases in Carcasses Not Condemned**	**Rate of Nephritis Cases in Carcasses Not Condemned**	**Total Number of Hogs Condemned Due to Nephritis**	**Rate of Condemnation Due to Nephritis**
Establishments known to have received hogs exposed to MC contaminate feed	6,618,760 [b]	332	0.5×10^{-4}	17	0.3×10^{-5}
All other establishments receiving hogs	31,044,701	18,027	5.8×10^{-4}	855	2.8×10^{-5}

[a] Data obtained from FSIS' electronic Animal Disposition Reporting System (eADRS) on 5/18/07.
[b] Note that only a small portion of all hogs sent to these establishments were exposed.

. **Table A-6: Analytical Results for Melamine and Melamine-Compounds in Swine Urine***

Location	Number of Samples Collected	Melamine	Cyanuric Acid
Location A [a,b]	7 samples last day of exposure	All positive Range <500 ppb to 940 ppb	Not Analyzed
	3 samples Day 1 after withdrawal	All positive Range 1,020 to 2,220 ppb	Not Analyzed
	8 samples Day 7 after withdrawal	1 detected < 500 ppb 7 Negative	Not Analyzed
Location B [c]	60	Results not Reported	Results not Reported
Location C [c]	6	473 ppb 2.5 ppm 3 ppm 2.2 ppm 882 ppb 362 ppb	Not Analyzed
Location D [c]	20	9 samples positive	5 samples positive
Location G [c]	7	531 ppb 321 ppb 237 ppb 184 ppb 823 ppb 203 ppb 266 ppb	Not Analyzed
Location H [c]	13	21 ppm 23 ppm 63 ppm 20 ppm 91 ppm 60 ppm 120 ppm 26 ppm 64 ppm 35 ppm 12 ppm 17 ppm 55 ppm	Not Analyzed
Location I [a]	7	All positive	Not Analyzed

[a] Analysis conducted by University of California at Davis.
[b] Urine analysis conducted daily from day of exposure to Day 14 post exposure. Six urine samples were analyzed daily on Day 8 through Day 14 post-withdrawal. One of the 6 samples from Day 9 had detectable levels of melamine (<500 ppb); all other of the 41 samples did not contain detectable levels of melamine.
[c] Analysis conducted at a private or state laboratory.

* Data are presented as concentrations in part per million (ppm), part per billion (ppb) or qualitatively as positive or negative, depending on results reported.

Appendix III

Farmed Fish Tissue and Fish Feed Sample Results

May 24, 2007

This appendix provides a summary of the investigations and analysis of farmed fish feed and tissue samples. FDA learned that a shipment mislabeled as wheat gluten was shipped from China to a manufacturer of pelleted fish feed in Canada, and that the pelleted fish feed was shipped to 199 consignees in the United States. The wheat gluten was from the same source as the contaminated wheat gluten used in the recalled pet food. The vast majority of the consignees were hatcheries operated by fishery or wildlife agencies for raising fingerlings or juveniles for stocking local ponds, streams, lakes, etc. Two aquaculture farms in the U.S. used the contaminated feed. FDA sampled edible fish tissue, as well as feed, from both farms. As indicated in the Table B-1below, none of the fish were found to contain melamine, at a lower limit of detection of 10 ppb. As shown in Table B-2, some of the feed samples did contain melamine.

Table B-1. Analysis of Fish Tissues for Melamine

Product	Results
Fish	Negative
Fish	Negative

Table B-2. Analysis of Fish Feed for Melamine

Product	Results
Fish Feed	Positive 125 ppm Melamine
Fish Feed	Positive 140 ppm Melamine
Fish Feed	Negative
Fish Feed	Negative
Fish Feed	Positive 400 ppm Melamine
Fish Feed	Positive 53 ppm Melamine 2.46 ppm Ammeline
Fish Feed	Positive 150 ppm Melamine