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Fauzia Aslam Rashid Date

Melamine exposure in US population

By

Fauzia Aslam Rashid

Master of Public Health

Environmental Health Department

_____

Dana Boyd Barr, Ph.D.
Committee Chair

_____

Paige Tolbert, Ph. D.
Committee Member

Melamine exposure in US population

By

Fauzia Aslam Rashid

Ph.D.

University of the Punjab, Lahore, Pakistan

1998

Thesis Committee Chair: Dana Boyd Barr, Ph.D.

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Abstract

Melamine exposure in US population
By
Fauzia Aslam Rashid

Background: Exposure to melamine and its analogue, cyanuric acid, occurs through a variety of commercial products like housewares, paints, plastic, papers, fabric, inks and many others. The intentional adulteration of pet food and infant formula with melamine has been reported to cause kidney damage, renal failure and death in certain cases. Co-exposures to melamine and cyanuric acid in livestock, pets and laboratory animals have shown higher toxicity compared with melamine and cyanuric acid alone. Melamine exposure remains to be a public health concern because of its tremendous use in daily life and the limited knowledge and understanding of its toxicity in humans. Our study objectives were to describe the population distribution of urinary melamine and cyanuric acid in the general US population, to evaluate their relation and to determine if doses calculated from the urinary values were within the FDA guidelines for exposure.

Methods: Data used for this study were collected as a part of the National Health and Nutrition Examination Survey (NHANES) for the years 2003-2004. Melamine and cyanuric acid were measured in a random 492-person subset of the entire survey maintaining representativeness of the sample to the US population. Three variables (age, sex, and racial/ethnicity) were evaluated for descriptive analysis for both melamine and cyanuric acid outcome and adjusted for urinary creatinine levels. Sex, age and race/ethnicity groups were compared to each other within the group by using the PROC GLM procedure. Statistical significance was established at $\alpha = 0.05$ significance level.

Results: No significant differences in melamine concentrations were observed among age, sex and racial/ethnic groups. However, we did see significantly higher cyanuric acid levels among males as compared to females ($p < 0.0001$). Adjusting for sex, age, race/ethnicity, and urinary creatinine, we found significantly lower melamine concentrations in non-Hispanic blacks as compared to non-Hispanic whites ($p=0.0031$) and in those younger than 20 years when compared to older participants ($p=0.0415$). Creatinine adjusted data also showed significantly lower cyanuric acid concentrations in non-Hispanic blacks as compared to non-Hispanic white ($p=0.0001$). Melamine and cyanuric acid doses were reconstructed by estimating the absorbed doses in our population sample. No significant differences in the doses estimated for sex, age and various race/ethnicity groups were observed.

Conclusion: Our findings suggest that male have higher exposure of melamine and cyanuric acid as compared to female population. Our sample population's exposure to melamine and cyanuric acid did not fall within a range of regulatory concern.

Keywords: Melamine. Cyanuric acid. US. Kidney.

Melamine exposure in US population

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Table of Contents

Introduction and background:

Melamine is an organic base and a trimer of cyanamide, with a 1,3,5-triazine skeleton. Like cyanamide, it contains 66% nitrogen by mass and, if mixed with resins, has fire retardant properties due to its release of nitrogen gas when burned or charred, and has several other industrial uses. Melamine is also a metabolite of cyromazine, a pesticide. It is formed in the body of mammals, that have ingested cyromazine.[1] It has been reported that cyromazine can also be converted to melamine in plants.[2][3]

Melamine is commercially synthesized from urea. Other by-products of the reaction include cyanuric acid, ammeline, and ammelide. Melamine is a versatile compound frequently used in manufacturing, agrochemical and food sectors. Melamine is combined with formaldehyde to produce Melamine resin, a very durable thermostatic plastic and Melamine foam, a polymeric cleanser. Melamine is one of the major components in Pigment Yellow 150, which is a widely used colorant in inks and plastics.

Other commercial products containing melamine include countertops, dry erase boards, fabric, glues, housewares, and dinner wares. Melamine and its salts are used as fire-retardant additives in paints, plastics, and paper.[4] Melamine derivatives of arsenical drugs are potentially important in the treatment of African trypanosomiasis.[5]

Back in 1958, melamine has been used in fertilizers [6] and also used as non-protein nitrogenous source for feeding the cattle. Later studies concluded it to be an ineffective non-protein source because of its slow hydrolysis in ruminants. [7]

Over the last decade, melamine has been added to animal and human foods to provide a non-protein nitrogen source that will produce a high protein content

measurement, which they desire, but is erroneous. Standard tests, such as the Kjeldahl and Dumas tests, estimate protein levels by measuring the nitrogen content, so they can be misled by added nitrogen-rich compounds such as melamine.

The intentional adulteration of pet food and infant formula with melamine has been reported to cause kidney damage, renal failure and death in certain cases. The mechanism of toxicity of melamine involves dose-dependent formation of crystals with either endogenous uric acid or the melamine metabolite, cyanuric acid (also a metabolite of triazine herbicides), in renal tubules resulting in potential acute kidney failure.

The toxicity of melamine and its analogue in humans and animals has been reported widely. The first outbreak of melamine toxicity occurred in pets in 2004 and 2007, when melamine tainted pet food caused severe renal failure and deaths in dogs and cats [8,9,10]. The pet food tested from this outbreak contained melamine and cyanuric acid. [9,10] The amounts of melamine found in some tested food ranged from 10 to 3,200 ppm. Various animal studies concerning sub-acute or chronic melamine exposure revealed stone formation. Incidence ranges from 5 to 100% depending on the dosage of melamine, gender, and amount of water intake. [11,12]

A study performed on B6C3F1 mice with exposure to 13 week of melamine showed that male mice are much more affected than females despite similar body weights. Relative risk of stone formation in males is twice as great as in females. [11]

Co-exposures to melamine and cyanuric acid in livestock, fish, pets and laboratory animals have shown higher toxicity compared with melamine and cyanuric acid alone. Several studies were done to evaluate the safety of melamine and cyanuric acid. In one study, cats were fed melamine, cyanuric acid, or both at increasing doses.

The cats fed only one compound had no renal failure or urine crystals at doses of 181 mg/kg body weight for melamine or at doses of 243 mg/kg body weight for cyanuric acid. However, the cats fed both compounds developed urinary stones at a dose of 32 mg/kg body weight of melamine and cyanuric acid. [13] Two other animal studies showed that melamine and cyanuric acid when administered together at 400 mg kg /day/ produced renal stones, but when they were administered separately, no stones formed. [14,9] Based on these studies, melamine and cyanuric acid appeared to be relatively safe in low doses when administered individually, but together resulted in the formation of stones.

Melamine caught the attention of a worldwide audience in 2008 when several incidences of kidney stones and kidney failure were reported in multiple regions of China. These problems occurred in children who consumed melamine-tainted milk. [15,16] An estimated 294,000 infants in China were affected, with more than 51,900 hospitalizations and 6 deaths. [16] Of the affected children, 99% were younger than 3 years old and developed symptoms 3 to 6 months after ingesting the formula, [17] their sole or major food source. Some of the children were asymptomatic; however, most symptoms included irritability, dysuria, difficult urination, renal colic, hematuria, or stone passage. [17] Hypertension, edema, or oliguria also occurred in more severe cases. Melamine can be detected in the urine of the affected children by biochemical test or the presence of fan shaped crystals. Analyses of stone composition mainly demonstrated melamine and uric acid. [18] Stones were radiolucent, and plain x-ray films failed to show their presence.

Levels of melamine in one company's line of infant milk formula products were up to 6,197 ppm of melamine, which was much higher than levels found in other milk

products and secondary contaminated foods. [16] In a 2009 study of 683 children diagnosed in Beijing in 2008 with nephrolithiasis and 6,498 children without nephrolithiasis aged < 3 years, investigators found that in children exposed to melamine levels < 0.2 mg/kg per day, the risk for nephrolithiasis was still 1.7 times higher than in those without melamine exposure, suggesting that the risk of melamine-induced nephrolithiasis in young children starts at a lower intake level than the levels recommended by the World Health Organization.[19]

Liu and his group from Beijing University published a follow up study reviewing ultrasound images of infants who fell ill in the 2008 contamination. They found that while most children in a rural Chinese area recovered, 12% still showed kidney abnormalities 6 months later. The potential for long-term complications after exposure to melamine remains a serious concern.[20]

The study by Guan *et al*. [21] demonstrated that infants exposed to the melamine formula with (>500 ppm melamine) were 7 times as likely to have stones as those exposed to melamine-free formula, and preterm infants were 4.5 times as likely to have stones as full-term infants. Urinalysis did not appear to be an adequate method of screening for melamine associated urinary stones. Biomarkers of early renal disease suggest that melamine-related stone formation appeared to be associated more with glomerular dysfunction rather than tubular dysfunction. Lam *et al*. [22] conducted a study in which they determined that urine melamine levels could be a biomarker of residual melamine load in the body, and found a strong correlation between renal stone size and

urinary melamine levels. However, there was no such correlation for cyanuric acid levels in urine.

Apart from the intentional adulteration of food products in Asia, melamine could potentially migrate from plastics. This is estimated to result in a baseline exposure of 0.0019–13 µg/kg b.w./day for melamine and 70 µg/kg b.w./day for cyanuric acid. [23] The Food and Drug Administration (FDA) and other regulatory agencies have published advisories for the acceptable levels of melamine in foods. According to FDA levels of melamine and its analogues below 2.5 ppm in foods other than infant formula do not raise public health concerns. In late 2008, the FDA detected melamine (0.137ppm) and cyanuric (0.247 ppm) in several samples of infant formula in the USA which were below the safety standard. The current safety standard for infant formula in the United States has been set to 1ppm based on a 3-kg infant ingesting 0.15 kg of powdered formula per day.

Although there are numbers of articles about melamine toxicity on different species of animals, a limited amount of knowledge is available about melamine toxicity in humans and its long term effects are unknown. Generally, the renal system is the main target for melamine toxicity. However, some other studies suggest melamine had other biological actions, like skin sensitivity, liver necrosis, cell apoptosis, carcinogenicity, genotoxicity, erythrocyte hemolysis and may lead to toxicity in immune and reproductive systems. It is well known that infants are more vulnerable to the toxicity of melamine because of underdevelopment of multiple organs, especially kidney and brain. Fetus and infant periods play the most fundamental role in brain development, including increase in

neuron number and synaptogenesis which are the physiological basis of learning and memory. It has been reported that melamine can pass through the placental barrier and blood-brain barrier and exerts toxic effects on the central nervous system. In experimental rats, melamine was found in different brain regions following exposure and could gradually increase with prolongation of exposed time. Melamine was found to enhance rat hippocampal CA_1 neurons. [24] Melamine was reported to impair spatial cognition and hippocampal synaptic plasticity by presynaptic inhibition of glutamatergic transmission in infant rats. [25]

Proper and timely management of any future outbreaks of melamine contamination of animal feed and human food will require access to state-of-the-art screening and confirmatory analytical methods to identify and quantify the presence of melamine and its related triazines, both in food items and in biological matrices. Many enzyme-linked immunosorbent assays (ELISA), liquid chromatography (LC), and gas chromatography (GC) techniques have been described in the literature[26,27,28] that have been successfully used to analyze these triazines in feed, blood, urine, and tissues. X-ray diffraction and Fourier transform infrared spectra of crystals formed in these matrices, together with mass spectrometry, have been used and can be used for identifying crystals and confirming their chemical composition in pets and human population. Besides testing for melamine, identification of reliable biomarkers of exposure and toxicity could provide improved diagnostic tools during the early stages of an outbreak. Quantifying the expression levels of selected biomarkers genes may become an important tool for identifying melamine and its analogs toxicity as reported by Camacho et al. [29]

Melamine is a very versatile material and its exposure continues to be a public health concern because of its tremendous use in manufacture, its potential use for feed adulteration and the limited understanding of its toxicity in humans.

Our study objectives were to describe the population distribution of urinary melamine and cyanuric acid in the general US population, to evaluate their relation and to determine if doses calculated from the urinary values were within the FDA guidelines for exposure.

Materials and methods:

Data Source

Data for this study were collected as a part of the National Health and Nutrition Examination Survey (NHANES) for the years 2003-2004, and is a part of the public release data set for NHANES.

NHANES is a program of studies conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC). This program is designed to assess the health and nutritional status of adults and children in the United States. The survey is unique in that it combines interviews and physical examinations. Melamine and cyanuric acid were measured in a random 492-person subset of the entire survey maintaining representativeness of the sample to the US population.

Health interviews were conducted in participant's homes and data were collected. Physical examinations were performed in specially-designed and equipped mobile centers. Blood and urine samples were collected during these examinations. Two screener modules were adopted to screen the participant's eligibility for taking part in the survey.

Urine sample collection

Blood and urine samples were collected from participants during the physical examination. Urine samples were collected from individuals aged 6 years and above. No exclusion criteria were applied to collect the urine samples.

Pre-examination procedures depended on the age and health of the individual examined. For example persons > 12 years of age were asked to fast for 2-12 hrs, depending upon appointment time, and persons with known diabetes or < 12 years of age were asked to eat a normal diet before the examination.

Demograhpic categories

Age was reported at the time of the household interview as the age in years at the last birthday. For statistical analysis age was categorized into < 20 yrs and ≤ 20 yrs. A race/ethnicity variable was created based on self-reported race and ethnicity. This variable defines three major racial/ethnic groups: non-Hispanic white, non-Hispanic black, and Mexican American. Persons who self-reported race as none of the above three racial/ethnic groups were not included in the study.

Laboratoy methods

Laboratory methods for detecting melamine and cyanuric acid in urine samples have been described in detail in previous studies. [30,31]

Dose construction:

Melamine and cyanuric doses were reconstructed based on the biomarkers values to determine the percentage of population who have exposures less than or equal to FDA

guidelines. Maximum estimated dose a person can have is 2.5 mg/Kg bw. A deterministic approach was used to back-calculate the absorbed dose assuming a steady state exposure. [32] Essentially, the person was assumed to excrete a constant amount of melamine or cyanuric acid in an average of 1500 mL urine per day. The value was divided by the participant's body weight to derive the dose.

Statistical analysis

Descriptive statistics of melamine and cyanuric acid was done with demographic variables by using SAS and SUDAAN softwares. Three variables (age, sex, and racial/ethnicity) were evaluated for both melamine and cyanuric acid outcome and adjusted for urinary creatinine levels. Sex, age and race/ethnicity groups were compared to each other within the group by using the PROC GLM procedure. Statistical significance was established at $\alpha = 0.05$ significance level.

Results:

Melamine and cyanuric acid were measured in a random 492 person subset of the entire survey representing the sample from US population for the year 2003-2004. The whole volume and creatinine-adjusted descriptive data for melamine are presented in Tables 1 and 2, respectively. The data are shown as total values and stratified by age, sex and race/ethnicity. Geometric means, medians and selected percentiles are shown. With a limit of detection (LOD) of 0.06 ng/mL, melamine was detected in 76% of the samples tested. Similar distribution data are shown for cyanuric acid in Tables 3 and 4. With an LOD of 0.09 ng/mL, the frequency of detection of cyanuric acid was 97%.

Table 1. Concentrations of melamine (ng/mL) in the general population (NHANES 2003-2004). Data in percentile estimates include confidence intervals and standard errors.

	N	GM	25th	50th	75th	90th	95th	FOD (%)
All	492	1.41	1.25 (NE-1.92) NE	2.85 (2.50-3.14) 0.15	4.35 (3.98-5.04) 0.25	8.44 (6.09-11.54) 1.28	11.90 (8.38-23.39) 3.52	100
Sex								
Male	231	1.56	1.69 (NE-2.23) NE	2.92 (2.59-3.17) 0.14	4.36 (3.86-4.90) 0.24	8.12 (5.85-12.17) 1.48	13.03 (8.43-NE) NE	47
Female	261	1.28	1.00 (NE-1.82) NE	2.57 (2.10-3.21) 0.26	4.33 (3.93-5.90) 0.46	8.96 (5.83-11.73) 1.38	11.69 (8.18-19.83) 2.73	53
Age (Yrs)								
≤ 20	168	1.05	NE (NE-2.05) NE	2.39 (1.98-2.76) 0.18	3.77 (3.05-4.81) 0.41	6.52 (4.21-13.18) 2.10	13.18 (5.38-NE) NE	34
≥ 20	324	1.55	1.47 (NE-1.96) NE	2.96 (2.57-3.23) 0.16	4.45 (4.08-5.29) 0.28	8.95 (6.53-11.51) 1.17	11.60 (8.72-27.56) 4.42	66
Race/Ethnicity								
Non-Hispanic White	209	1.57	1.48 (NE-2.04) NE	2.72 (2.39-3.11) 0.17	4.24 (3.86-4.92) 0.25	8.26 (5.63-10.64) 1.17	11.17 (7.63-NE) NE	42.5
Non-Hispanic Black	133	1.05	NE (NE-1.81) NE	2.93 (2.10-3.43) 0.31	4.55 (3.61-5.36) 0.41	9.25 (6.51-11.68) 1.21	11.87 (8.03-20.00) 2.81	27
Mexican American	150	1.17	NE (NE-2.38) NE	2.98 (2.22-3.86) 0.38	4.61 (3.38-7.94) 1.07	10.69 (4.63-16.64) 2.82	14.12 (6.22-21.36) 3.55	30.5

NE= not estimated

Table 2. Concentrations of melamine (μg/g creatinine) in the general population (NHANES 2003-2004). Data in percentile estimates include confidence intervals and standard error.

	N	GM	25th	50th	75th	90th	95th	FOD (%)
All	492	1.25	0.84 (0.04-1.59) 0.36	2.46 (2.26-2.76) 0.12	5.49 (4.19-6.52) 0.55	11.02 (7.89-18.11) 2.40	18.50 (10.97-32.09) 4.95	100
Sex								
Male	231	1.11	0.89 (0.04-1.66) 0.38	2.26 (1.63-2.55) 0.22	3.62 (2.83-5.22) 0.56	7.19 (5.50-18.07) 2.95	13.16 (7.17-22.49) 3.59	47
Female	261	1.40	0.69 (0.04-1.78) 0.41	2.85 (2.46-3.31) 0.20	6.72 (5.42-9.09) 0.86	13.30 (9.60-22.26) 2.97	21.51 (13.26-34.80) 5.05	53
Age (Yrs)								
≤ 20	168	0.78	0.05 (0.02-1.22) 0.28	1.99 (1.31-2.35) 0.24	3.32 (2.63-5.47) 0.67	7.69 (5.23-12.470 1.70	12.66 (7.15-NE) NE	34
≥ 20	324	1.44	1.17 (0.05-1.68) 0.38	2.69 (2.32-2.92) 0.14	5.90 (4.62-7.30) 0.63	12.53 (7.96-18.48) 2.47	18.65 (11.06-32.49) 5.03	66
Race/Ethnicity								
Non-Hispanic White	209	1.47	1.17 (0.06-1.69) 0.38	2.51 (2.31-2.89) 0.14	5.54 (4.06-7.73) 0.86	12.95 (7.70-20.99) 3.12	21.16 (10.49-36.15) 6.02	42.5
Non-Hispanic Black	133	0.68	0.04 (0.02-0.94) 0.22	2.11 (1.28-2.66) 0.32	3.82 (3.00-5.73) 0.64	7.17 (4.47-10.43) 1.40	12.05 (7.02-15.84) 2.07	27
Mexican American	150	1.02	0.06 (0.03-2.23) 0.52	2.54 (1.41-3.04) 0.38	5.84 (2.95-6.51) 0.83	9.16 (5.93-15.12) 2.16	13.15 (6.56-NE) NE	30.5

NE= not estimated

Table 3. Concentrations of cyanuric acid (ng/mL () in the general population (NHANES 2003-2004). Data in percentile estimates include confidence intervals and standard error.

	N	GM	25th	50th	75th	90th	95th	FOD (%)
All	452	5.73	3.90 3.57-4.36) 0.18	5.69 (5.20-6.44) 0.29	9.01 (7.98-9.83) 0.43	14.00 (11.06-18.04) 1.64	19.33 (14.25-21.05) 1.59	100
Sex								
Male	217	6.79	4.41 (3.93-5.31) 0.32	6.49 (5.53-8.06) 0.59	9.72 (8.07-12.41) 1.02	15.19 (10.84-19.49) 2.03	20.20 (13.43-50.17) 8.62	48
Female	235	4.86	3.59 (2.90-4.15) 0.29	5.21 (4.36-5.83) 0.34	8.02 (6.63-9.15) 0.59	11.98 (9.56-14.91) 1.26	15.56 (11.93-19.06) 1.67	52
Age (yrs)								
<20	153	6.50	4.36 (3.08-5.35) 0.53	5.90 (5.24-7.95) 0.64	10.55 (9.7-14.39) 1.51	16.73 (12.72-19.56) 1.60	18.91 (15.02-21.45) 1.51	34
≥ 20	299	5.51	3.86 (3.32-4.30) 0.23	5.63 (4.91-6.29) 0.32	8.62 (7.41-9.42) 0.47	12.89 (10.60-15.38) 1.12	19.26 (12.35-25.42) 3.07	66
Race/Ethnicity								
Non-Hispanic White	198	5.84	3.89 (3.22-4.52) 0.30	5.72 (4.95-6.88) 0.45	9.08 (8.00-10.00) 0.47	13.75 (10.82-19.28) 1.98	19.30 (13.34-21.05) 1.81	44
Non-Hispanic Black	129	5.83	3.82 (3.27-4.79) 0.36	5.62 (5.14-6.62) 0.35	9.21 (7.89-10.88) 0.70	13.73 (10.63-22.72) 2.83	23.33 (12.11-NE) NE	28.5
Mexican American	125	5.33	4.06 (3.27-4.78) 0.35	5.47 (4.70-6.57) 0.44	7.96 (6.02-13.23) 1.69	14.82 (8.44-16.31) 1.85	15.65 (11.09-NE) NE	27.5

NE= not estimated

Table 4. Concentrations of cyanuric acid (µg/g creatinine) in the general population (NHANES 2003-2004). Data in percentile estimates include confidence intervals and standard error.

	N	GM	25th	50th	75th	90th	95th	FOD (%)
All	452	4.97	3.25 (2.89-3.78) 0.21	5.11 (4.51-5.73) 0.29	8.25 (7.15-10.15) 0.70	13.94 (11.83-16.97) 1.21	18.34 (16.02-23.02) 1.64	100
Sex								
Male	217	4.79	3.16 (2.88-3.56) 0.16	4.73 (4.26-5.62) 0.32	7.43 (6.59-8.550 0.46	11.33 (8.43-13.94) 1.29	14.76 (12.54-28.90) 3.84	48
Female	235	5.15	3.40 (2.47-4.37) 0.45	5.47 (4.54-6.70) 0.51	10.21 (7.55-11.89) 1.02	16.24 (12.96-18.65) 1.34	19.36 (16.09-25.14) 2.12	52
Age (Yrs)								
≤ 20	153	4.75	2.97 (1.68-4.15) 0.58	4.98 (4.23-6.74) 0.59	7.75 (6.76-11.03) 1.00	11.96 (7.69-16.11) 1.98	14.66 (8.22-NE) NE	34
≥ 20	299	5.04	3.28 (2.92-4.10) 0.27	5.15 (4.47-5.90) 0.34	8.28 (7.09-10.36) 0.77	14.86 (11.91-17.13) 1.22	19.07 (15.58-24.96) 2.20	66
Race/Ethnicity								
Non-Hispanic White	198	5.35	3.40 (2.88-4.54) 0.39	5.29 (4.61-6.86) 0.53	8.73 (7.12-11.18) 0.95	15.62 (11.85-17.77) 1.39	18.83 (14.81-26.09) 2.65	44
Non-Hispanic Black	129	3.82	2.72 (2.17-3.11) 0.22	4.16 (3.31-5.15) 0.43	6.29 (5.10-7.77) 0.62	9.74 (7.72-13.01) 1.24	12.95 (9.75-14.41) 1.09	28.5
Mexican American	125	4.55	3.17 (2.46-4.07) 0.38	4.50 (4.24-5.91) 0.39	7.18 (6.26-9.57) 0.78	12.90 (8.09-19.75) 2.74	19.10 (13.01-22.04) 2.12	27.5

NE= not estimated

Melamine was not correlated with cyanuric acid as would be expected since the latter has multiple sources. No significant differences in melamine concentrations were observed among age, sex and racial/ethnic groups as shown in the Figure 1. However, we did see significantly higher cyanuric acid levels among males as compared to females ($p<0.0001$; Figure2).

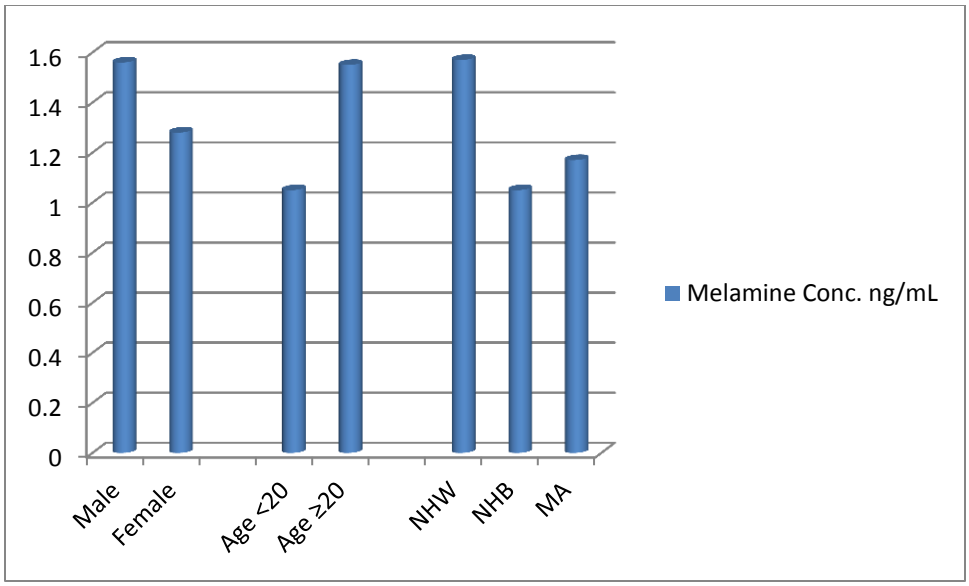

Figure 1: Geometric mean concentration of Melamine (ng/mL) for each sex, age and race/ethnicity group.

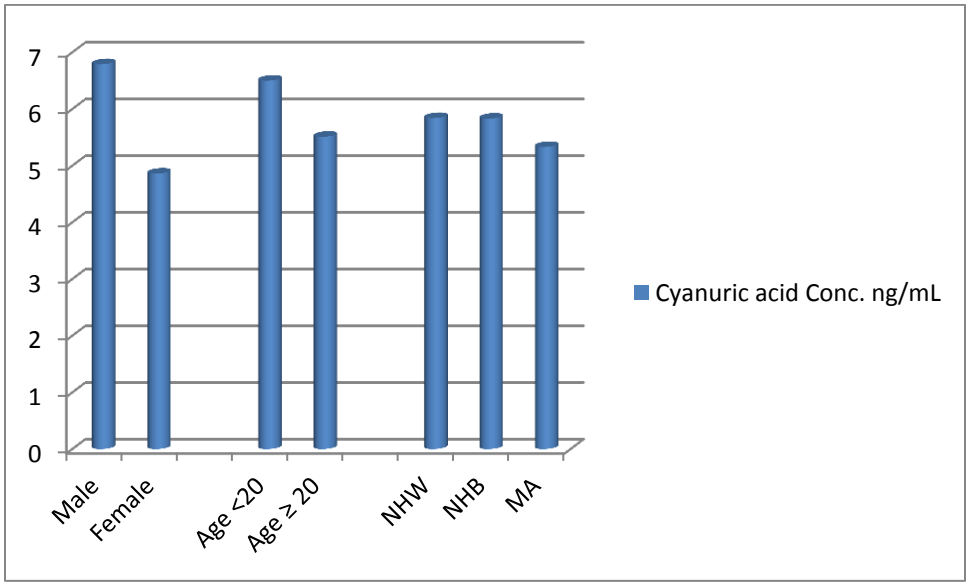

Figure 2: Geometric mean concentration of cyanuric acid (ng/mL) for each sex, age and race/ethnicity group.

Least squares geometric means were calculated using log10 transformed concentration data as the dependent variable adjusting for sex, age, race/ethnicity, and urinary creatinine. The data showed significantly lower melamine concentrations in non-Hispanic blacks as compared to non-Hispanic white ($p=0.0031$; Figure 3) and in those younger than 20 years when compared to older participants ($p=0.0415$; Figure 3).

Creatinine adjusted data also showed significantly lower cyanuric acid concentrations in non-Hispanic blacks as compared to non-Hispanic white (p=0.0001; Figure 4). We did not observe any significant difference in cyanuric acid levels in other groups (Figure 4).

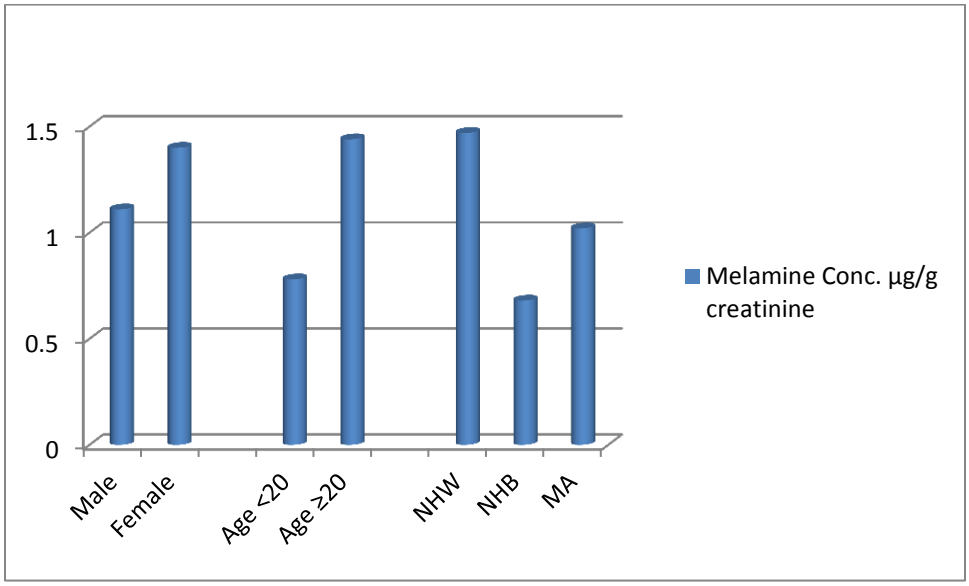

Figure 3: Geometric mean concentration of creatinine adjusted Melamine (µg/g creatinine) for each sex, age and race/ethnicity group.

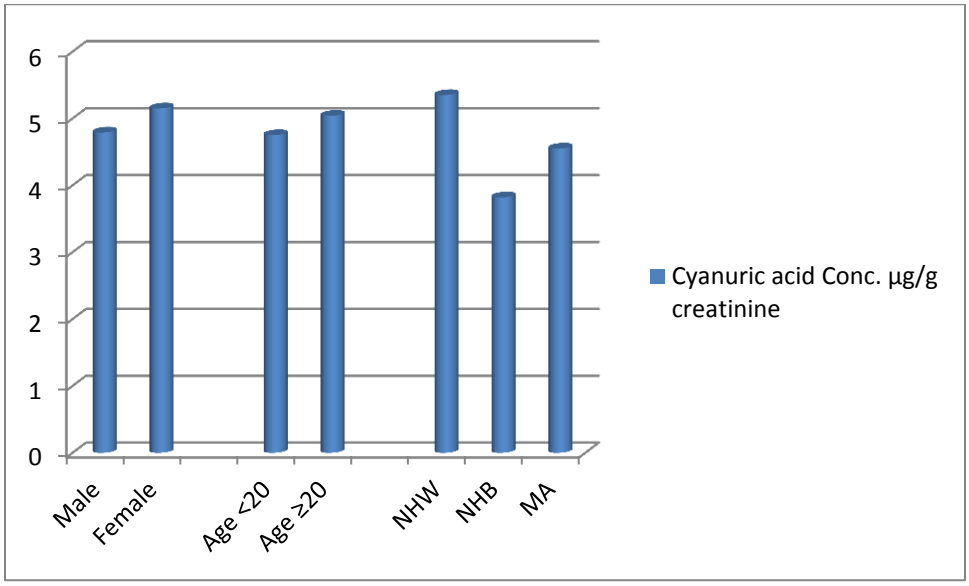

Figure 4: Geometric mean concentration of creatinine adjusted cyanuric acid (µg/g creatinine) for each sex, age and race/ethnicity group.

Melamine and cyanuric acid doses were reconstructed by estimating the absorbed doses in our population sample categorized into sex, age and race/ethnicity groups (Table 5-8). We did not see any significant differences in the doses estimated for sex, age and various race/ethnicity groups. Overall the representative sample for US population which we used for our analysis did not show melamine or cyanuric acid levels above the FDA guidelines (Tables 5-8).

Table 5. Melamine dose (ng/Kg bw) in the general population (NHANES 2003-2004). Data in percentile estimates include confidence intervals and standard error.

	N	GM	25th	50th	75th	90th	95th	FOD (%)
All	486	26.75	19.55 (1.06-34.17) 7.77	52.18 (46.26-57.03) 2.53	84.15 (76.61-104.13) 6.46	165.81 (119.65-259.42) 32.79	265.82 (160.25-458.74) 70.02	
Sex								
Male	227	26.73	22.90 (0.94-39.19 8.97	49.53 (40.51-57.55) 4.0	72.98 (63.68-87.81) 5.66	155.65 (95.78-243.40) 34.63	265.32 (153.74-625.05) 110.56	46.7
Female	259	26.76	16.85 (1.08-34.36) 7.81	54.30 (44.40-63.28) 4.43	95.50 (82.96-119.70) 8.62	173.22 (118.86-268.23) 35.04	264.10 (155.58-407.17)59.02	53.3
Age (yrs)								
<20	171	23.91	1.38 (1.00-43.98) 10.08	54.82 (41.82-62.14) 4.77	86.95 (61.49-126.18) 15.18	166.78 (142.33-303.69) 37.85	294.46 (160.67-NE) NE	35.2
≥ 20	315	27.69	27.03 (1.04-34.24) 7.79	50.63 (43.39-55.25) 2.78	83.28 (76.93-107.81) 7.24	162.58 (116.74-259.04) 33.38	260.82 (151.79-463.87) 73.21	64.8
Race/Ethnicity								
Non-Hispanic White	209	29.68	27.46 (2.03-35.67) 7.89	50.18 (42.49-54.51) 2.82	81.39 (71.43-103.03) 7.41	153.44 (115.60-265.84) 35.24	260.86 (152.37-559.82) 95.58	43
Non-Hispanic Black	128	19.11	0.92 (0.76-31.59) 7.23	52.24 (35.52-60.58) 5.88	95.22 (75.44-114.11) 9.07	171.52 (117.24-205.94) 20.81	241.58 (176.79-NE) NE	26.3
Mexican American	149	22.73	1.06 (0.84-46.19) 10.64	57.60 (39.93-75.02) 8.23	95.50 (67.05-150.27) 19.52	171.64 (112.55-286.94) 40.91	278.12 (129.11-NE) NE	30.7

NE=not estimated

Table 6. Melamine dose (µg/g creatinine/Kg bw) in the general population (NHANES 2003-2004). Data in percentile estimates include confidence intervals and standard error.
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	N	GM	25th	50th	75th	90th	95th	FOD (%)
All	486	23.78	13.57 (0.98-24.55) 5.53	43.27 (39.40-47.90) 1.99	103.42 (83.07-137.43) 12.75	234.69 (165.46-296.99) 30.85	373.28 (241.23-611.81) 86.93	
Sex								
Male	227	19.10	13.88 (o.79-24.93) 5.66	38.93 (27.54-43.04) 3.64	64.29 (51.50-92.66) 9.66	151.98 (100.72-281.45	260.08 (132.93-475.150 80.28	46.7
Female	259	29.24	12.59 (0.85-31.06) 7.09	61.57 (44.30-75.63) 7.35	151.47 (105.81-190.54) 19.88	297.11 (200.94-404.40) 47.73	433.25 (297.11-903.36) 142.22	53.3
Age (yrs)								
<20	171	17.92	1.70 (0.48-28.38) 6.54	38.53 (30.08-44.58) 3.40	98.87 (73.89-133.30) 13.94	183.00 (131.01-369.69) 55.99	359.07 (172.21-NE) NE	35.2
≥ 20	315	25.94	17.74 (1.14-26.43) 5.93	44.19 (40.32-50.08) 2.29	101.97 (82.82-159.810 18.06	248.91 (160.67-353.71) 45.28	370.09 (217.28-624.26) 95.47	64.8
Race/Ethnicity								
Non-Hispanic White	209	27.88	16.35 (2.14-28.06) 6.08	43.31 (38.21-51.22) 3.05	104.89 (81.40-169.22) 20.60	274.71 (162.88-398.01) 55.16	417.61 (223.34-700.55) 111.94	43
Non-Hispanic Black	128	12.89	0.68 (0.43-17.22) 3.94	37.25 (22.91-56.95) 7.98	79.37 (47.51-118.59) 16.67	132.94 (97.59-220.75) 28.89	225.82 (128.92-NE) NE	26.3
Mexican American	149	19.68	1.20 (0.74-39.11) 9.00	44.92 (21.53-72.63) 11.99	104.43 (76.35-153.80) 18.17	188.52 (119.82-286.31) 39.06	260.40 (162.41-NE) NE	30.7

NE=not estimated

Table 7. Concentrations of Cyanuric acid dose (ng/Kg bw) in the general population (NHANES 2003-2004). Data in percentile estimates include confidence intervals and standard error.

	N	GM	25th	50th	75th	90th	95th	FOD (%)
All	486	108.03	67.04 (43.67-80.06) 8.54	98.48 (85.49-120.82) 8.29	165.39 (138.14-187.25) 11.52	289.60 (207.92-384.39) 41.40	390.91 (289.83-532.11) 56.83	100
Sex								
Male	227	115.93	72.33 (56.16-88.58) 7.61	99.28 (87.20-137.17) 11.72	163.35 120.19-210.31) 21.14	294.18 (179.72-347.56) 39.37	356.28 (220.95-649.26) 11.47	46.7
Female	259	100.90	56.66 (35.37-76.68) 9.69	96.76 (74.95-119.52) 10.46	166.06 (132.26-194.40) 14.58	278.77 (185.31-423.01) 55.76	422.19 (259.45-544.33) 66.83	53.3
Age (Yrs)								
< 20	171	145.33	81.13 (51.03-112.760 14.48	127.29 (94.32-173.92) 18.67	254.06 (167.34-413.86) 57.83	436.24 (307.48-946.63) 149.93	584.70 (383.60-NE) NE	35.2
≥ 20	315	98.33	64.85 (41.34-76.84) 8.33	90.35 (79.52-113.73) 8.02	149.43 (119.53-172.96) 12.54	216.85 (117.55-297.90) 29.64	302.55 (208.44-368.510 37.55	64.8
Race/Ethnicity								
Non-Hispanic White	209	110.43	66.24 (40.22-86.34) 10.82	99.17 (82.91-131.94) 11.50	161.58 (132.84-198.61) 15.43	301.36 (202.75-384.63) 42.67	388.74 (289.28-687.53) 93.42	43
Non-Hispanic Black	128	102.20	64.89 (55.17-70.97) 3.71	95.44 (81.49-117.300 8.40	165.86 (148.78-181.220 7.61	277.38 (209.12-417.21) 48.81	345.29 269.76-NE) NE	26.3
Mexican American	149	103.58	72.52 (41.38-87.27) 10.76	95.05 (82.81-111.63) 6.76	168.79 (115.49-219.87) 24.48	234.24 (176.14-392.24) 50.69	366.83 (222.05—NE) NE	30.7

NE=not estimated

Table 8. Cyanuric acid dose (µg/g creatinine/Kg bw) in the general population (NHANES 2003-2004). Data in percentile estimates include confidence intervals and standard error.

	N	GM	25th	50th	75th	90th	95th	FOD (%)
All	486	94.08	49.63 (30.24-65.81) 8.34	90.08 (69.52-112.03) 9.97	161.15 (125.65-209.66) 19.71	292.62 (229.26-377.32) 34.73	476.45 (289.64-651.05) 84.78	100
Sex								
Male	227	82.44	48.77 (35.25-55.77) 4.81	78.65 (63.53-105.68) 9.89	132.77 (108.05-152.840 10.51	209.55 (163.28-258.43) 22.32	304.56 (229.70-473.70) 57.24	46.7
Female	259	106.90	49.70 (22.01-82.90) 14.28	106.52 (73.52-137.62) 15.04	213.45 (153.49-279.29) 29.51	363.87 (245.97-522.61) 64.89	527.60 (320.38-743.98) 99.37	53.3
Age (yrs)								
< 20	171	106.64	49.54 (31.68-79.28) 11.17	112.70 (68.73-155.61) 20.38	182.39 (149.76-297.53) 34.66	328.44 (181.77-1104.83) 216.53	930.97 (227.80-NE) NE	35.2
≥ 20	315	90.42	49.63 (28.54-65.04) 8.56	88.89 (64.83-108.09) 10.15	145.61 (113.49-188.14) 17.51	279.65 (226.66-355.77) 30.29	429.07 (283.59-515.80) 54.47	64.8
Race/Ethnicity								
Non-Hispanic White	209	101.14	49.71 (25.94-84.72) 13.79	98.22 (66.69-133.62)15.70	168.61 (122.32-233.91) 26.18	313.74 (229.52-442.33) 49.92	484.08 (271.67-856.06) 137.09	43
Non-Hispanic Black	128	69.52	37.87 (30.40-49.70) 4.53	71.59 (54.96-97.51) 9.98	138.37 (90.11-160.50) 16.51	191.41 (141.05-248.15) 25.12	242.40 (178.20-NE) NE	26.3
Mexican American	149	88.17	51.55 (29.46-65.54) 8.46	78.11 (63.85-115.31) 12.07	125.41 (121.07-174.26) 12.48	268.72 (169.23-519.32) 82.13	480.68 (243.08-585.27) 80.27	30.7

NE=not estimated

Discussion:

Our study presents a population-based descriptive- analysis of urinary melamine and its metabolite, cyanuric acid, in the US population stratified by demographic variables. Our results suggest that males had higher levels of both melamine and cyanuric acid than females although it was only significantly different for the latter. This is particularly important as some of the previous studies in mice demonstrated greater toxicity in male mice than female mice, despite their relatively similar body weights. The relative risk of stone formation was found to be twice as high as compared to females. [11]

When we took into account urinary creatinine variations to correct for melamine and cyanuric acid data in demographic groups as previously reported [33], we found significant differences in both melamine and cyanuric acid levels among non-Hispanic blacks compared to the non-Hispanic white population. These differences may be attributable to urinary creatinine -variations between the two races. However, we can't rule out the possibility for food preference variations among various race/ethnic groups.

We estimated individual doses from urinary metabolite concentrations of melamine and cyanuric acid with assuming a steady state exposure, for sex, age and race/ethnic groups.[32] These estimated doses were then compared to FDA established reference dose set by FDA guidelines.[15] We did not observe melamine and cyanuric acid levels equal or higher than the FDA standard of 2.5 mg/kg body weight. The findings indicate that our sample population's exposure to melamine and cyanuric acid did not fall within a range of regulatory concern.

Conclusion and future directions:

While there are not much data available from direct human studies on the effect of melamine, data from animal studies can be used to predict adverse health effects. Generally, the renal system is the main target for melamine toxicity. However, some other studies suggest melamine had other biological actions, like toxicity in immune and reproductive systems and may pose a potential carcinogenic risk.

We plan to extend our studies further to evaluate the relation between urinary melamine and kidney dysfunction by taking into consideration all the factors contributing towards melamine-induced nephrotoxicity and renal failure in US population.

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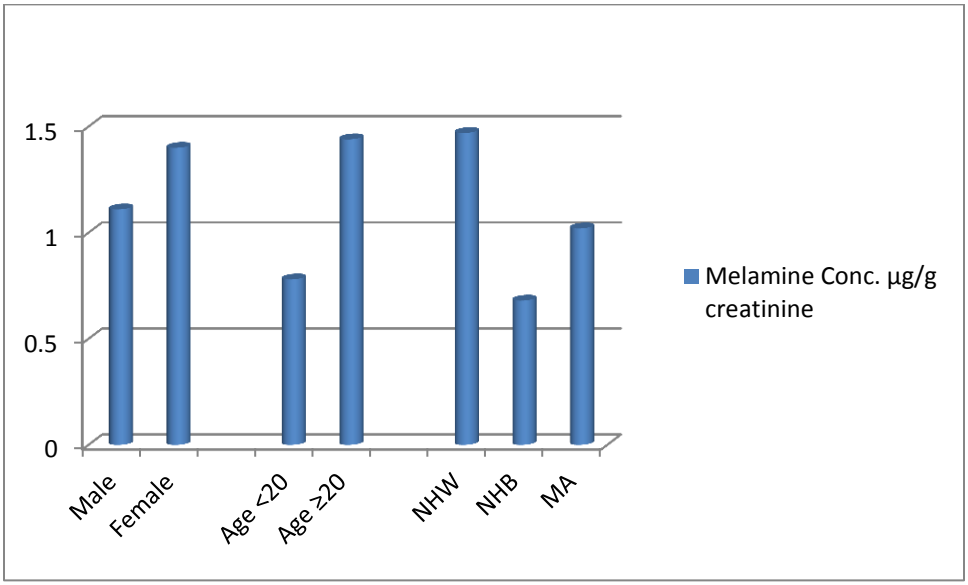

Figure 2: Geometric mean concentration of creatinine adjusted Melamine (µg/g creatinine) for each sex,

age and race/ethnicity group.