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High melamine migration in daily-use melamine-made tableware

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ABSTRACT

Melamine is commonly used to manufacture tableware, and this could be one of the important exposure sources in humans. The study aims to measure melamine migrated from different material-made tableware by the most sensitive technique of liquid chromatography–tandem mass spectrometry (LC–MS/MS). The test samples were filled with pre-warmed designated-temperature (from room temperature (∼20 °C), 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C, to 90 °C) simulant (either distilled water or 3% acetic acid) up to 20 ml and immersed in a water bath at that designated temperature for 15 or 30 minutes (min). High melamine migration levels, ranging from 6.97 to 19.03 μg/ml, can be measured from all melamine-made samples containing 20 ml 3% acetic acid in water bath of 90 °C for 30 min, whereas melamine cannot be detectable in all other material-made samples in the same condition. In addition, the cheaper the melamine-made tableware samples, the higher the melamine migration levels. The migration of melamine amount is dependent on different temperatures, contact times, simulant, and prices of tableware. Since tableware is used in daily life, it is prudent to cautiously select materials that contain foodstuffs.

1. Introduction

The outbreak of melamine-tainted formula milk has raised concern about melamine toxicity on adverse renal outcomes in children worldwide in 2008 [1]. In adults, we recently reported that melamine exposure measured by urinary melamine levels was also associated with both uric acid and calcium urolithiasis [2]. Because of the uncertainty of the safety of melamine dose in human intake, both the World Health Organization (WHO) and the US Food and Drug Administration (FDA) recommended to lower the tolerable daily intake (TDI) of melamine from 0.5 mg per kg body weight per day (mg/kg bw/day) to 0.2 mg/kg bw/day and from 0.63 mg/kg bw/day to 0.063 mg/kg bw/day, respectively [3,4]. However, Chen et al. subsequently reported that several children with melamine-related urolithiasis had an intake even below the updated WHO or FDA recommended TDI [5]. Li and his colleagues also found that children exposed to melamine levels below 0.2 mg/kg per day (WHO recommended TDI) still have a 1.7 times higher risk of developing urolithiasis than those without melamine exposure in 683 renal stone children patients and 6498 children controls [6]. All these studies in children [1] and ours in adults [2] suggest that even exposure to low levels of melamine may be a risk factor for urolithiasis formation.

In addition to adding melamine purposely into infant formula to fallibly increase protein content [1], melamine is also popular to be used in the manufacturing of a variety of tableware for daily use, because of its cheap and relatively light and unbreakable characteristics [7]. A few studies have reported that melamine can migrate out of melamine-made tableware under the condition of high temperature or acid solution [8–11]. However, melamine levels in these studies were measured by a high performance liquid chromatog-

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raphy equipped with diode array detector (HPLC-DAD), which may not be sensitive enough, since previous studies in both children and adults have suggested potential nephro-urological hazards in low melamine exposure [1,2]. In addition, the daily uses of melamine-made tableware become very popular nowadays, and its quality, composition, and price have changed rapidly in the past years. Thus, we attempt to measure melamine levels using the most sensitive technique of liquid chromatography–tandem mass spectrometry (LC–MS/MS) in tableware made from different materials, especially melamine resin, in a wide range of temperatures. Our findings will add additional information as to how to regulate melamine-made table- or kitchen-ware in daily use.

2. Experimental

2.1. Samples

All tableware, including 5 different brands and sizes of melamine-made cups (MEL-1, -2, -3, -4, and -5) and one brand of each stainless steel-, polypropylene-, polycarbonate-, ceramics-, glass-, paper-made cups and disposable plastic cups (Fig. A), was purchased between 2009 and 2010 from local markets or retail shops in Kaohsiung City, which is in southern Taiwan. The price of MEL-1, MEL-2, and MEL-3 was about one US dollar per sample, whereas that of MEL-4 and MEL-5 was about 5 US dollars per sample. The total test number of each brand of melamine-made cups we purchased had 4–10 samples and the rest of other material-made cups had 3 (Table 1). To reduce experimental variation, all internal surfaces of tableware were white or gray and were not painted with any pictures. In addition, we confirmed the composition of one test sample of MEL-1 and MEL-2 to contain melamine by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) (Fig. B).

2.2. Chemicals

Melamine (1,3,5-triazine-2,4,6-triamine, $C_3H_6N_6$, CAS number 108-78-1) with purity 99% and its respective isotopically labeled homologues, $^{13}C_3{}^{15}N_3$-melamine (99% $^{13}C_3$ and 98% amino-$^{15}N_3$), as internal standard were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). 99.7% acetic acid (CH_3COOH) and 100.0% methanol (CH_3OH) were purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). A 100 ml 3% acetic acid solution was made by adding 3 ml 99.7% acetic acid to 97 ml distilled water. 99.9% HPLC-graded acetonitrile (C_2H_3N) was obtained from Fisher Scientific (Loughborough, UK). Formic acid (HCOOH) was obtained from J.T. Baker (Phillipsburg, NJ, USA). ~98% ammonium acetate (CH_3COONH_4) was from Merck-Schuchardt (Hohenbrunn, Germany).

2.3. Migration test

Each tableware sample was filled with the pre-warmed designated-temperature simulant (distilled water or 3% acetic acid) up to 20 ml and immersed in a water bath at that designated temperature for 15 or 30 minutes (min). The designated temperatures ranged from room temperature (~20 °C), 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C, to 90 °C. The orders of experiments of all tableware samples were from the lowest temperature to the highest one, and half of different brands and materials of tableware samples were used for the experiments of distilled water and the other half for the experiments of 3% acetic acid. After completing one experiment, all tableware test samples were thoroughly washed by distilled water, gently wiped by dry and clean cotton textiles, and reused for the next experiment. In each experiment, we used a glass beaker as our blank sample and treated it as the same condition as melamine-made tableware samples. To reduce the experimental variation, all test samples were reused less than 10 times; the averaged reused times were 8 for melamine-made tableware samples and 3 for other materials-made tableware samples.

To examine whether the melamine migration levels were similar in the brand new and used (have done ~8 repeated experiments before) melamine-made tableware samples, we filled 20 ml 90 °C distilled water in 3 new and 3 used MEL-1 samples and immersed them in a water bath at 90 °C for 30 min. This experiment was duplicated. Another 3 new and 3 used MEL-1 samples were also used in the same experiment of 3% acetic acid simulant. These experiments were also applied to new and used MEL-2 samples. We found overall mean melamine levels were similar in the new and used tableware samples of both MEL-1 and MEL-2 experiments (Fig. C).

2.4. LC–MS/MS analysis

The melamine levels in simulant were measured by an Agilent 1200 HPLC (Agilent Technologies, Palo Alto, CA, USA) coupled with an API 4000Q triple-quadrupole mass spectrometry (API 4000QTrap, Applied Biosystems/MDS SCIEX, Concord, Canada) and an electrospray ionization (ESI) source in a positive ion mode [2]. A 10 μl sample was injected into a Phenomenex Luna HILIC column (100 mm × 2 mm, 3 μm, Torrance, CA, USA) at a flow rate of 250 μl/min in the isocratic mode. The mobile phase was set at 5% of solution A (2 mM ammonium acetate with 0.2% formic acid in water) and 95% of solution B (2 mM ammonium acetate with 0.2% formic acid in acetonitrile). Multiple reaction monitoring (MRM) mode was used with the characteristic fragmentation transitions m/z 127 → 85 of melamine and m/z 133 → 89 of the internal standard for quantification and m/z 127 → 68 of melamine was used for confirmed quantitative analysis with the dwell time set at 100 ms and unit resolution (Fig. 1). The Turbo Ion-Spray source was run at a temperature of 550 °C with the following settings: curtain gas, 25 (arbitrary units); source gas 1, 50; source gas 2, 55; collision activated dissociation (CAD) gas pressure, medium; ion spray voltage, 5500. Product ions of m/z 85 and 68 for melamine were obtained using collision energy (CE) = 27 and 43, respectively, declustering potential (DP) = 81, collision exit potential (CXP) = 4, and entrance potential (EP) = 10. Product ions of m/z 89 for internal standard were obtained using CE = 27, DP = 66, CXP = 6, and EP = 10. The limit of quantitation (LOQ) defined by a signal-to-noise (S/N) of 10 from the chromatograms of sample spiked standard was 0.005 μg/ml.

2.5. Method validation

The validation experiments were done in 3% acetic acid at 90 °C for 30 min. $^{13}C_3{}^{15}N_3$-melamine was used as the internal standard (IS) for calibration at concentrations ranging from 5, 10, 20, 50, 100, 200, 500 to 1000 ppb (1 ppb = 0.001 μg/ml) with a correlation coefficient (R^2) of 0.992 in mobile phase solutions. Quantification of the calibration concentrations in validation experiments was within 15% of the theoretical value with a CV less than 15% analyzed on different days (Fig. D).

The averaged recovery rate (±SD) of 8 repetitions at three designated concentrations of 5, 50 and 500 ppb was 72.58% (±0.84), 75.81% (±4.38), and 84.46% (±37.54), respectively. Table 2 shows the accuracy and precision (coefficient of variance (CV)) of 8 repetitions, which were 95.39% and 6.99% in 5 ppb, 103.35% and 3.59% in 50 ppb, and 99.66% and 0.10% in 500 ppb. The intra- and inter-day relative standard deviations (RSDs) ranged from 0.34–4.61% to 0.25–5.43%, respectively, with replicated analysis (n = 8) of a spiked sample ranging 5, 50, or 500 ppb.

Table 1
Characteristics of different material-made tableware samples (cups) in the migration experiments.

Sample materials	Number of samples	Diameter (dm)	Depth (dm)	ISA[b] (dm^2)
MEL[a]-1	10	0.52	0.09	0.34
MEL-2	10	0.44	0.11	0.30
MEL-3	8	0.60	0.07	0.41
MEL-4	4	0.64	0.07	0.46
MEL-5	4	0.48	0.09	0.32
Stainless steel	3	0.66	0.06	0.47
Polypropylene	3	0.57	0.08	0.40
Polycarbonate	3	0.50	0.09	0.34
Ceramics	3	0.61	0.09	0.46
Glass	3	0.58	0.12	0.48
Paper	3	0.53	0.10	0.39
Plastic	3	0.45	0.13	0.34

[a] Melamine.
[b] Inner surface area.

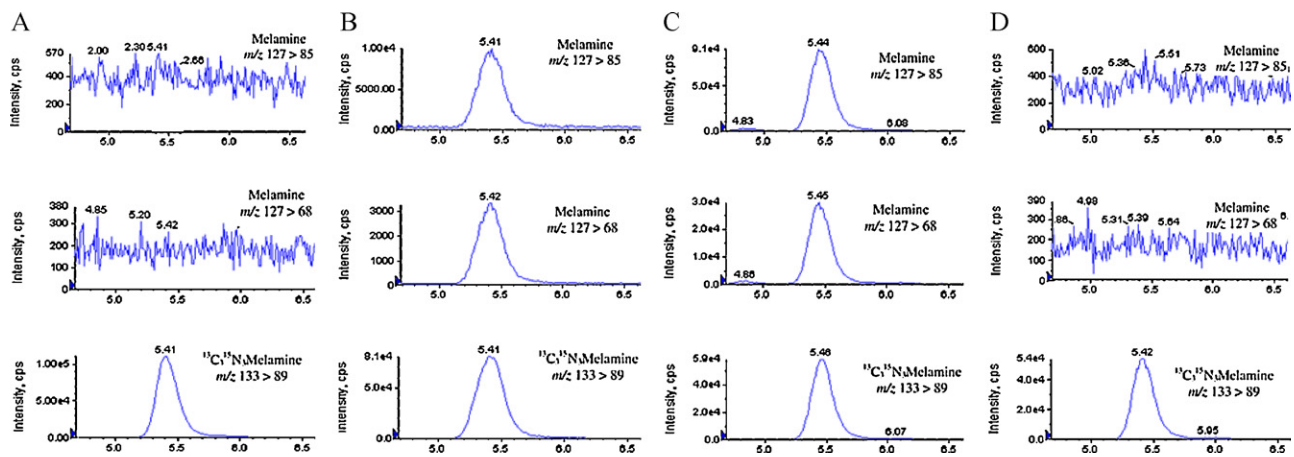

Fig. 1. Melamine signals of ion chromatograms for (A) blank in distilled water; (B) standard (0.005 μg/ml melamine) in mobile phase; (C) pre-warmed 90 °C distilled water in a MEL-1 tableware sample and immersed it in a water bath at the same temperature for 30 min (3.69 μg/ml); (D) pre-warmed 90 °C 3% aqueous acetic acid in a stainless steel tableware sample and immersed it in a water bath at the same temperature for 30 min (non-detectable) (upper and middle panels of chromatograms are melamine signals and lower panel of the chromatogram is $^{13}C_3{}^{15}N_3$-melamine internal standard (IS)).

2.6. Statistical analysis

ANOVA statistics with Bonferroni correction was used to compare melamine migration levels in different temperatures. Student *t*-test was used to compare melamine migration levels in different simulants (distilled water *vs.* 3% acetic acid), contact times (15 min *vs.* 30 min), or prices. The Pearson coefficient was used to investigate the correlation between different temperatures and melamine migration levels in the same melamine resins and treatment as well as between the price of melamine-made test samples and the migration amount of melamine. The data were analyzed using the SPSS statistical software version 14.0; all *p*-values were two-sided with significance at <0.05.

3. Results

3.1. Melamine migration levels in different material-made tableware samples

High melamine levels, ranging from 6.97 to 19.03 μg/ml, were measured from all melamine-made tableware samples containing 20 ml 3% acetic acid that were immersed in a water bath at 90 °C for 30 min. In contrast, all melamine migration levels in other material-made tableware samples such as stainless steel-, polypropylene-, polycarbonate-, ceramics-, glass-, paper-made cups and disposable plastic cups were below LOQ in the same condition (data not shown). Thus, the subsequent experiments were focused on melamine-made samples at different temperatures and contact times.

Table 2
Accuracy and precision of three designated melamine validation solutions ($n = 8$).

	Day 1		Day 2		Accuracy[b]		Inter-day difference (%)	Intra-day difference (%)
	Mean	RSD[a] (%)	Mean	RSD (%)	Day 1	Day 2		
5 ppb[c]	5.04	6.99	4.77	9.92	100.87	95.39	5.43	4.61
50 ppb	51.38	3.59	51.68	2.84	102.75	103.35	0.59	3.35
500 ppb	499.53	1.00	498.30	0.91	99.91	99.66	0.25	0.34

[a] RSD (relative standard deviation or precision) = SD/mean.
[b] Accuracy = (mean observed concentration/standard concentration) × 100.
[c] ppb = 0.001 μg/ml.

3.2. Melamine migration levels in new and used melamine-made tableware samples and different amounts of simulant in melamine-made tableware samples

To compare whether the melamine migration levels in cups were similar in a volume of 20 ml simulant and ~150 ml simulant (filled simulant up to 1 cm from the rim of cups), we randomly selected 3 of 10 MEL-1 samples and 3 of 4 MEL-5 samples for this experiment and immersed them in a water bath at 90 °C for 30 min. Each sample was done this experiment twice. We found mean (±SD) melamine migration levels (μg/ml) from MEL-1 samples were similar in 20 ml and ~150 ml distilled water (2.68 ± 0.19 *vs.* 2.52 ± 0.40, $p = 0.30$) ($n = 6$). The similar result of MEL-1 samples was found in 3% acetic acid (24.04 ± 0.88 *vs.* 22.76 ± 1.61, $p = 0.10$). The similar melamine migration levels were also found in the experiments of MEL-5 samples (20 ml *vs.* ~150 ml simulant, μg/ml; in distilled water: 1.81 ± 0.29 *vs.* 1.92 ± 0.55, $p = 0.63$; in 3% acetic acid: 10.68 ± 0.86 *vs.* 12.36 ± 1.868, $p = 0.06$) ($n = 6$). Since the melamine migration levels were similar in these two different amounts of simulant, we used 20 ml simulant for the subsequent experiments.

3.3. Melamine migration levels in different melamine-made tableware samples

In the experiments of different melamine-made tableware samples containing 20 ml distilled water or 3% acetic acid in different temperatures of water bath for 30 min, we found that the higher the temperature, the more the melamine migration levels in simulant of five different brands of melamine-made tableware samples (Tables 3 and 4). Except melamine migration levels in some repeated experiments of MEL-4 and MEL-5 with distilled water at the lowest temperatures (~20 °C and 30 °C) were non-detectable (below LOQ, 0.005 μg/ml), in all melamine-made tableware at relatively high temperature (≥40 °C) can be detected the migration of melamine. The number of non-detectable melamine migration was 6 and 7 of 8 repeated experiments in MEL-4 and in MEL-5 with distilled water, respectively, at room temperature and 2 of 8 repeated experiments in MEL-5 with distilled water at 30 °C. In addition, migration of melamine was consistently and significantly higher in melamine-made tableware samples when containing 3% acetic acid, compared to distilled water in all different temperatures of water bath for 30 min ($p = 0.001$ in room temperature and $p < 0.001$ from 30 °C to 90 °C).

In order to be comparable in different sizes of different brands of melamine-made tableware samples, we corrected melamine levels by the inner surface area (ISA) and found that the higher the temperature, the more melamine migration in all five different brands of samples filled with either distilled water or 3% acetic acid simulant (Table 4).

3.4. Correlation between the price of tableware samples and melamine migration levels

The melamine levels migrated from MEL-1, MEL-2 and MEL-3 were higher than from MEL-4 and MEL-5 at all temperatures and both simulant, especially in 3% acetic acid (Tables 3 and 4). The Pearson correlation coefficients between the price of tableware samples and the migration amount of melamine in the temperatures from ~20 °C, 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C, to 90 °C were −0.57, −0.42, −0.69, −0.63, −0.73, −0.35, −0.75 and −0.43 in distilled water, and −0.90, −0.92, −0.93, −0.92, −0.478, −0.55, −0.52 and −0.51 in 3% acetic acid (data not shown). All correlations were statistically significant (all p-values < 0.05). In addition, the melamine migration levels in the cheapest melamine-made tableware samples (MEL-3) were significantly higher than the most expensive ones (MEL-4) in all temperatures and both simulants (all p-values < 0.05 in distilled water and all p-values < 0.001 in 3% acetic acid from ~20 °C to 90 °C; Fig. 2).

3.5. Melamine migration levels at different contact times in melamine-made tableware samples

Noodle soups are one of the most popular foods in Taiwan and the rest of Asia. In Taiwan, the majority of restaurants or vendors use melamine-made tableware to contain hot noodle soups. To examine the cooling rate of hot noodle soups, we measured temperature change by the interval of 5 min for 30 min in three different kinds of hot noodle soups contained in melamine-made bowls (~700 ml) ordered randomly from three different restaurants. We found that the initial mean temperature (±SD) was 67.67 °C (±1.53 °C). After 15 and 30 min, the mean temperatures (±SD) were still 54.01 °C (±2.03 °C) and 46.33 °C (±2.52 °C), respectively (Fig. E).

Furthermore, to evaluate whether the contact time (30 min *vs.* 15 min) can affect the release of melamine, we measured melamine migration levels in simulant of melamine-made tableware samples containing 20 ml distilled water or 3% acetic acid at two different temperatures (50 °C and 90 °C). We found that melamine migration levels were significantly higher when contact time was 30 min *vs.* 15 min in both simulant in the temperature condition of 50 °C (mean ± SD = 0.13 ± 0.02 μg/ml *vs.* 0.10 ± 0.02 μg/ml, p-value = 0.001 in distilled water and 0.51 ± 0.18 μg/ml *vs.* 0.38 ± 0.15 μg/ml, p-value = 0.004 in 3% acetic acid) (Fig. 3). The significant results were even striking in a 90 °C temperature condition (2.46 ± 0.90 μg/ml *vs.* 0.67 ± 0.22 μg/ml in distilled water and 12.08 ± 4.08 μg/ml *vs.* 5.29 ± 0.60 μg/ml in 3% acetic acid, both p-values < 0.001).

4. Discussion

This study found that melamine migration levels can be measured from all melamine-made tableware samples, but not other tableware samples made from materials such as stainless steel-, polypropylene-, polycarbonate-, ceramics-, glass-, paper-made and (disposable) plastic cups. The migration amount of melamine is dependent on different temperatures, contact time, simulant, and prices. Melamine migration levels in previous similar studies were measured by either HPLC/UV–VIS or HPLC/UV, of which the melamine detection limit is not as sensitive as LC–MS/MS used in this study [8–12]. The first six studies reported from Japan, Philippines, England, and Denmark found that various melamine amounts were measured from different brands and sizes of melamine-made tableware test samples either in water or in 3% or 4% acetic acid simulant [8–11]. After the outbreak of melamine-tainted formula milk in China, Lu et al. [12] measured melamine migration levels in 13 polypropylene-made samples, 3 polycarbonate-made samples, and 6 melamine-formaldehyde-made samples and 15 diary product packages, which were all bought from supermarkets in Beijing, China, or collected from dairy companies, at 60 °C for 2 h with water, 3% acetic acid, n-hexane, or 15% ethanol. The melamine migration levels were measured by HPLC-UV/VIS and it was found that only 3 out of 6 melamine resin containers had detectable levels of migration. Melamine levels could not be detected in the rest of the samples. Because the melamine detection limit in that study was 0.18 μg/ml, which was at least one order higher than ours (0.005 μg/ml), no detectable melamine levels in the test samples from the study of Lu et al. [12] did not mean no residual melamine was migrated from containers. Their test samples are commonly used in our daily life. Since the studies in humans, particularly in children, have already suggested that even exposure to low levels of melamine may be a potential

Table 3
Melamine migration levels in different melamine resin samples at different temperatures and simulant (μg/ml).[a]

		MEL-1	MEL-2	MEL-3	MEL-4	MEL-5
RT	DW[b]	0.02 ± 0.01[d]	0.03 ± 0.001	0.03 ± 0.01	–[e]	–[f]
	AA[c]	0.16 ± 0.01	0.14 ± 0.03	0.13 ± 0.03	0.01 ± 0.01	0.01 ± 0.001
30 °C	DW	0.05 ± 0.01	0.04 ± 0.01	0.08 ± 0.01	0.03 ± 0.004	–[g]
	AA	0.24 ± 0.02	0.22 ± 0.02	0.18 ± 0.07	0.05 ± 0.01	0.04 ± 0.02
40 °C	DW	0.06 ± 0.01	0.05 ± 0.004	0.09 ± 0.01	0.04 ± 0.003	0.02 ± 0.01
	AA	0.27 ± 0.03	0.29 ± 0.02	0.28 ± 0.05	0.02 ± 0.01	0.07 ± 0.03
50 °C	DW	0.07 ± 0.003	0.11 ± 0.02	0.14 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
	AA	0.37 ± 0.09	0.41 ± 0.05	0.40 ± 0.07	0.14 ± 0.03	0.12 ± 0.03
60 °C	DW	0.37 ± 0.02	0.42 ± 0.05	0.57 ± 0.07	0.08 ± 0.01	0.10 ± 0.03
	AA	0.65 ± 0.10	0.64 ± 0.02	1.75 ± 0.22	0.46 ± 0.04	0.16 ± 0.06
70 °C	DW	0.40 ± 0.02	0.65 ± 0.04	1.20 ± 0.04	0.36 ± 0.05	0.43 ± 0.05
	AA	0.90 ± 0.07	2.43 ± 0.06	2.94 ± 0.19	1.64 ± 0.06	0.85 ± 0.13
80 °C	DW	0.75 ± 0.03	1.34 ± 0.12	1.28 ± 0.09	0.71 ± 0.05	0.57 ± 0.04
	AA	12.13 ± 0.65	3.51 ± 0.58	9.98 ± 0.73	1.80 ± 0.04	0.99 ± 0.25
90 °C	DW	3.43 ± 0.15	1.93 ± 0.04	2.18 ± 0.07	0.86 ± 0.10	2.04 ± 0.10
	AA	19.03 ± 0.80	9.69 ± 0.49	16.80 ± 0.43	7.40 ± 0.70	6.97 ± 0.40

[a] 8 repeated experiments for 30 min in each brand of melamine-made tableware.
[b] Distilled water.
[c] 3% acetic acid.
[d] Mean ± standard deviation.
[e] 6 of 8 repeated experiments were below LOQ (limit of quantitation).
[f] 7 of 8 repeated experiments were below LOQ.
[g] 2 of 8 repeated experiments were below LOQ.

risk factor for urolithiasis formation [1,2], their conclusion about the release of melamine from melamine resin containers can be ignored should be cautiously judged.

Melamine migration levels in this study were significantly higher than those in the previous ones [8–12], especially in the experiments of 3% acetic acid. It was probably due to melamine-made tableware samples in different studies were manufactured in different years or different countries which influenced the composition of tableware samples and impacted the migration amount of melamine.

This study showed that melamine migration levels from MEL-1, MEL-2 and MEL-3 were constantly higher than those from MEL-4 and MEL-5 in the experiments at all temperatures and simulant. All test samples were purchased from local markets or retailed shops and the samples of MEL-1, MEL-2, or MEL-3 were much cheaper than those of MEL-4 and MEL-5 (∼1 *vs.* ∼5 US dollars per sample). The different prices of melamine-related table- or kitchen-ware in the market may be due to the different manufacturing processes or purity of materials which are possibly associated with the quality of the table- or kitchen-ware and might influence the migration amount of melamine. Making higher quality table- or kitchen-ware and preventing it from contacting high temperatures can decrease melamine exposure in humans.

Lund and Petersen suggested that the melamine migrated from melamine-made tableware samples were probably from two sources: melamine released from the first several exposures in simulant is from residual monomers of new and non-used samples and subsequent exposures are from the degradation of the polymer of samples. Although the mechanisms of melamine release are different for different exposure times, they found that mean melamine migrations (mg/dm^2) in 6 specimens of one test plate sample in 3% acetic acid at 95 °C for 30 min were similar up to an exposure number of 10 (the 1st exposure = 0.34, $n = 1$; the 2nd exposure = 0.30 ± 0.06, $n = 5$; the 10th exposure = 0.34 ± 0.09, $n = 6$)

Table 4
Melamine migration levels corrected by ISA ($\mu g/dm^2$) in different melamine resin samples at different temperatures and simulants.[a]

		MEL-1	MEL-2	MEL-3	MEL-4	MEL-5
RT	DW[b]	0.06 ± 0.02[d]	0.08 ± 0.03	0.08 ± 0.01	–[e]	–[f]
	AA[c]	0.48 ± 0.03	0.45 ± 0.09	0.31 ± 0.07	0.03 ± 0.03	0.05 ± 0.03
30 °C	DW	0.141 ± 0.02	0.12 ± 0.04	0.19 ± 0.02	0.07 ± 0.01	–[g]
	AA	0.72 ± 0.04	0.72 ± 0.06	0.43 ± 0.17	0.11 ± 0.02	0.11 ± 0.07
40 °C	DW	0.18 ± 0.02	0.17 ± 0.01	0.20 ± 0.03	0.09 ± 0.01	0.05 ± 0.02
	AA	0.82 ± 0.09	0.96 ± 0.06	0.67 ± 0.11	0.13 ± 0.02	0.21 ± 0.08
50 °C	DW	0.22 ± 0.01	0.37 ± 0.08	0.33 ± 0.02	0.11 ± 0.01	0.19 ± 0.02
	AA	1.09 ± 0.25	1.36 ± 0.17	0.96 ± 0.16	0.30 ± 0.06	0.38 ± 0.10
60 °C	DW	1.12 ± 0.04	1.39 ± 0.16	1.37 ± 0.16	0.17 ± 0.02	0.31 ± 0.08
	AA	1.96 ± 0.29	2.12 ± 0.07	4.22 ± 0.52	1.00 ± 0.09	0.51 ± 0.20
70 °C	DW	1.21 ± 0.08	2.15 ± 0.14	2.88 ± 0.10	0.77 ± 0.10	1.37 ± 0.16
	AA	2.70 ± 0.19	7.98 ± 0.20	7.09 ± 0.46	3.55 ± 0.12	2.67 ± 0.41
80 °C	DW	2.24 ± 0.13	4.41 ± 0.38	3.09 ± 0.21	1.54 ± 0.11	1.79 ± 0.14
	AA	36.37 ± 1.70	11.53 ± 1.89	24.06 ± 1.77	3.89 ± 0.09	3.11 ± 0.78
90 °C	DW	10.30 ± 0.57	6.35 ± 0.14	5.25 ± 0.156	1.85 ± 0.22	6.45 ± 0.31
	AA	57.17 ± 4.04	31.85 ± 1.60	40.52 ± 1.03	15.99 ± 1.50	22.03 ± 1.23

[a] 8 repeated experiments for 30 min in each brand of melamine-made tableware.
[b] Distilled water.
[c] 3% acetic acid.
[d] Mean ± standard deviation.
[e] 6 of 8 repeated experiments were below LOQ.
[f] 7 of 8 repeated experiments were below LOQ.
[g] 2 of 8 repeated experiments were below LOQ.

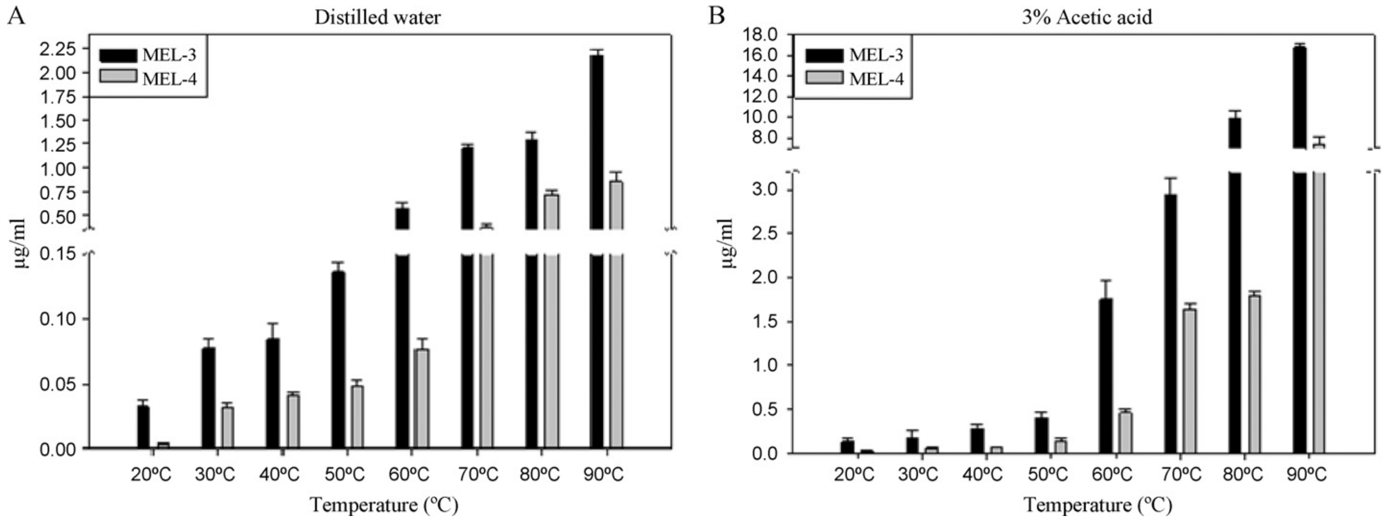

Fig. 2. Comparison of melamine migration levels in melamine-made tableware samples with the cheapest price (MEL-3) *vs.* the most expensive one (MEL-4) in 20 ml simulant (distilled water or 3% acetic acid) at the designated temperature for 30 min (mean ± SD, $n = 8$ repeated experiments, all p-values < 0.05 in distilled water and all p-values < 0.001 in 3% acetic acid).

[9]. They also found that melamine continued to be migrated-out from melamine-made test cups even used for several years in a day nursery. Our study also found melamine migration levels were pretty similar in both new and used melamine-made test samples in repeated experiments up to 6 exposure times (Fig. C). In order to reduce experimental variations, we used different test samples for the experiments of different simulants and the orders of experiments in different temperatures were from the lowest one to the highest one. In addition, up to 10 repeated experiments per test sample were performed in this study to avoid the effect of acidic corrosiveness. We also used cotton towels to softly and gently wipe the inside of the tableware for the next experiment in the same simulant to decrease the chance of scratching.

Melamine is a necessary additive material to make flame retardants, glues, and plastics [7]. Besides adding melamine purposely into infant formula milk to fallibly increase protein content, melamine was also commonly found in foodstuffs, including chicken eggs, fish, and vegetable crops imported from China [13,14]. Our findings, along with others, that daily-use melamine-made table- or kitchen-ware has a high potential to release melamine adds another source of melamine exposure in our lifetime [8–11]. These findings are consistent with the comments from Ingelfinger that melamine-contaminated food is more pervasive than was originally thought [15].

Until now, it is not clear how much melamine is safe for human intake, and whether long-term low levels of melamine intake may confer a healthy risk to humans [15]. The TDI originally recommended by WHO and US FDA was all based on selected animal toxicity assay. Although WHO and FDA have lowered their TDIs [15], two subsequent research studies have reported that some children with melamine-related urolithiasis had an intake even below the updated WHO or FDA recommended TDI [5]. Recently, Hsieh et al. challenged WHO and FDA TDI derived from lowest-observed-adverse-effect level (LOAEL) rather than non-observed-adverse-effect level (NOAEL) [16,17]. Thus, they further recommended that a lower TDI of melamine to 0.0081 mg/kg bw/day should be considered in humans [16]. Based on this TDI recommendation of 0.0081 mg/kg bw/day, assuming an average person weighs 60 kg (∼0.48 mg/day), using the MEL-3 test sample to contain hot noodle soup in a volume of 700 ml at the initial mean temperature of 60 °C for 15 min and the subsequent mean temperature of 50 °C for

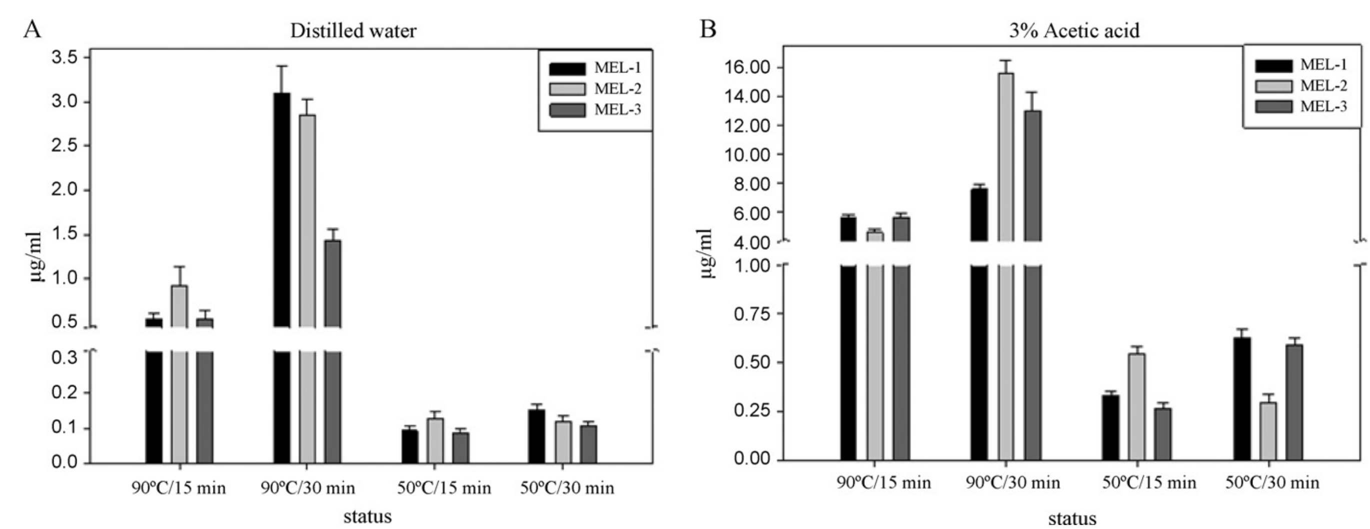

Fig. 3. Melamine migration levels in three different brands of melamine-made samples treated with pre-warmed 50 °C or 90 °C distilled water (DW) or 3% acetic acid (AA) at the same designated temperature for 15 or 30 min ($n = 4$ repeated experiments).

another 15 min (~30 min in total to consume one bowl of hot noodle soup), the intake of melamine amount will be approximately 0.75 mg, which exceeds this TDI recommendation ((1.75 μg/ml + 0.40 μg/ml)/2 × 700 ml/1000 μg/mg = 0.75 mg) (Table 3).

The Comité Européen de Normalisation (CEN) method [18] recommended that the melamine migration experiments be tested in four different simulants, including distilled water, 3% acetic acid, 15% ethanol and a fatty simulating food. This study only used distilled water and 3% acetic acid as simulant to test melamine migration from tableware. However, our experiments in distilled water and in 3% acetic acid representative for pH value higher than 6 of foodstuffs and sour foods can cover most container conditions for daily food products. Since melamine migration levels were not significantly different in the test samples with 20 ml or ~150 ml simulant, we chose the former one for most of the experiments in this study. In addition, melamine migration levels were also corrected by contact area, they were able to compare with the ones from the earlier studies. We did not know the actual composition of melamine resins in our test samplers. Thus, we are unable to examine the relationship between the contents of melamine in tableware and their migration of melamine concentrations.

5. Conclusions

Melamine migration can be detectable from daily-use melamine-made tableware, even in the low temperatures (30–40 °C). The migration amount of melamine sharply increases after 60–70 °C. The cheaper the melamine-made tableware samples, the higher the melamine migration levels. Although the migration amount of melamine is not large at low temperatures (<50 °C), it is important to realize that no adverse sub-chronic renal effect in relatively high melamine exposure cannot absolutely guarantee the health safety of low and continuous melamine exposure. Since tableware is used in daily life, it is prudent to cautiously select materials that contain foodstuffs.

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Conflict of interest

None. The sponsors had no role in the design and conduct of the study; in the collection, management, analysis, and interpretation of the data; and in the preparation, review, and approval of the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.01.128.

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