DOI:10.1021/jf904205y

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Deposition of Melamine in Eggs from Laying Hens Exposed to Melamine Contaminated Feed

Yiqiang Chen, Wenjun Yang, Zongyi Wang, Yong Peng, Bin Li, Liying Zhang,* and Limin Gong*

State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China

The deposition profile of melamine was studied in eggs obtained from laying hens fed melamine contaminated feed. A total of 180 laying hens were divided into five groups and were fed diets spiked with 0, 5, 25, 50, or 100 mg of melamine per kg of feed. Eggs collected on days 1, 3, 5, 7, 9, 11, 13, and 15 were analyzed by a gas chromatography−mass spectrometry method, which was fully validated for melamine analysis prior to use. For each treatment group, the melamine level in the eggs was similar from day 1 to day 15 ($P > 0.05$), suggesting that laying hens did not accumulate melamine for later deposition in eggs. The average melamine concentrations in eggs were 0.00 (below limit of detection), 0.16, 0.47, 0.84, and 1.48 mg/kg for the 0, 5, 25, 50, and 100 mg/kg treatment groups, respectively, which demonstrated an apparent dose−response relationship, and a safety threshold of 164 mg/kg melamine in the feed of laying hen was estimated when a maximum tolerance level of 2.5 mg/kg melamine in egg was adopted. These results provide a scientific basis for the risk assessment of melamine in feeds fed to laying hens.

KEYWORDS: Melamine; deposition; feed; eggs; gas chromatography−mass spectrometry

INTRODUCTION

Melamine (1,3,5-triazine-2,4,6-triamine) is a triazine-based chemical intermediate commonly used in the manufacture of amino resins, plastics and flame retardants (*1*). It received considerable public attention after the pet food contamination scandal in North America in 2007 and the melamine-tainted-milk powder incident in China in 2008 (*1*).

Melamine by itself has a relatively low toxicity with an oral LD_{50} of 3160 mg/kg in the rat (*2*) and is also unlikely to be a mutagen (*3*). However, it can bind with its analogues, such as cyanuric acid, to form crystals and then may induce significant renal toxicity and carcinogenic effects (*4*). Considering its potential toxicity, The United States Food and Drug Administration (FDA) has recommended a tolerable daily intake of 0.063 mg/kg body weight (BW)/day for melamine (*5*). Thus, for the protection of consumer health, it is essential to keep melamine residues below permitted levels in animal products designed for human consumption.

Melamine is not approved for use as a feed or food additive. However, it has been added illegally into feedstuffs or food to artificially distort their crude protein content. Melamine could also appear in feeds in a variety of ways such as the through the degradation of cyromazine (a feed additive used to control flies in barns), through the use of melamine as a binding agent in pelleted feeds and through migration from the environment into feedstuffs (*1*). Therefore, for risk assessment purposes, it is essential to investigate the relationship between the melamine level in feed and the level in animal-origin foods.

In previous studies, Andersen et al. (*6*) reported melamine residues in fish dosed with melamine at 400 mg/kg BW/day. In addition, Bermudez et al. (*7*) determined the melamine level in the tissues of broiler chickens fed diets with several concentrations of melamine (*7*). To our knowledge, it has not been established whether or not melamine contamination of diets fed to laying hens will increase melamine residues in eggs. Therefore, this study was conducted to assess the melamine deposition kinetics in eggs from laying hens exposed to different concentrations of dietary melamine.

MATERIALS AND METHODS

Chemicals and Reagents. Melamine (purity, 99%+) was purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile and methanol (HPLC grade) were supplied by Fisher Scientific (Fair Lawn, NJ). The derivatization reagent Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was purchased from Supelco (Bellefonte, PA). Concentrated ammonium hydroxide (w/v, 25%), trichloroacetic acid (TCA) and lead acetate were of analytical grade and were obtained from Beijing Reagent Corporation (Beijing, China). Purified water was prepared with a Milli-Q system (Millipore, Bedford, MA).

Animal Experiment. This experiment was approved by the Animal Welfare Committee of China Agricultural University and was carried out in the Nutrition and Metabolism Laboratory of the Ministry of Agricultural Feed Industry Center (Beijing, China). One hundred eighty White Leghorn laying hens (22 weeks of age and 1.5 kg BW) were divided into five groups in a completely randomized block design experiment. There

*Corresponding authors. L. Zhang: tel, +86-106-273-3588; fax, +86-106-273-3688; e-mail, zhangliying01@sina.com. L. Gong: tel, +86-106-273-3764; fax, +86-106-273-3688; e-mail, glm0213@126.com.

 Published on Web 02/24/2010

were 6 replicates for each group, and each replicate was composed of a battery of three cages with two hens in one cage (0.64 m^2). The hens of the five groups were fed a standard corn−soybean meal diet with feed contaminated with 0, 5, 25, 50, or 100 mg/kg melamine respectively. The four levels of melamine contaminated feeds were obtained by premixing melamine with feed followed by further mixing in blender. For each level, the melamine concentrations from six sampling points were determined to be around the target level with relative errors less than 10%.

The experiment lasted 15 days. All the hens were given free access to feed and water and were monitored daily for general health by qualified personnel. The room temperature was maintained between 20 and 25 °C with the relative humidity set at 60−70%. The birds were provided with 16 h of light per day with an intensity of 50 lx. Eggs were collected daily during the study. Each egg was labeled indicating date, group and cage number and stored at +4 °C. The eggs collected on days 1, 3, 5, 7, 9, 11, 13, and 15 were then analyzed for melamine concentration. Data analysis was performed by GLM procedures of SAS 9.0 (SAS Inst., Inc., Cary, NC) using the LSMEANS statement and the PDIFF option.

Sample Preparation. Eggs obtained from the above experiment were crushed, and the contents of the eggs from the same replicates were combined into a 150 mL conical flask. After the egg yolk and white were homogenized with a PowerGen 700D homogenizer (Fisher Scientific), 2 g of the homogenate was weighed into a 50 mL polystyrene centrifuge tube. Twenty milliliters of 2% trichloroacetic acid and 1 mL of 1% lead acetate solution were then added successively. The mixture was mixed with a vortex (IKA MS#, IKA Werke GmbH & Company, Deutschland, Germany) for 1 min, followed by sonication extraction for 20 min (KQ 5000, Kunshan Ultrasonic Apparatus Company, Kunshan, Jiagsu Province, China). After the solution was centrifuged at 8000 rpm for 10 min, the supernatant was subjected to solid phase extraction (SPE).

An Oasis MCX Cartridge (60 mg, 3 mL, Waters, Milford, MA) was used to clean up the extracts. The cartridge was preconditioned with 3 mL of methanol and 3 mL of water. Then, 3 mL of the supernatant obtained above was added into the cartridge which was allowed to flow at a rate of about 1 mL/min. The SPE column was washed with 3 mL of water and 3 mL of methanol and dried by applying vacuum for 1 min. Finally, the target chemical was eluted from the column with 3 mL of 5% (v/v) ammonium hydroxide/methanol into a glass vial by gravity. The eluate was evaporated to dryness under a gentle stream of nitrogen gas in a water bath at 50 °C. One hundred microliters of acetonitrile and 100 μL of BSTFA with 1% TMCS were added accurately into each vial, and the mixture was vigorously vortexed (IKA Werke GmbH, Deutschland, Germany) for 30 s. The vials were tightly capped and placed at 70 °C for 40 min to allow the silylation reaction to take place. The final solution was transferred into a vial with a liner and was used for gas chromatography−mass spectrometry (GC−MS) analysis.

GC−MS. Analysis of melamine was performed on an Agilent 6890 plus gas chromatograph equipped with a 7683 series auto sampler and an Agilent 5973N mass selective detector (Agilent Technologies, Santa Clara, CA). The gas chromatograph was fitted with a DB-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) from Agilent J & W Scientific (Folsom, CA). Helium was used as the carrier gas at a flow rate of 1 mL/min. The samples were injected using a splitless mode and the injection volume was 1 μL. The inlet temperature was at 250 °C, and the transfer line heater was set at 280 °C. The oven was maintained at the initial temperature of 75 °C for 1 min, heated to 300 °C at a rate of 30 °C/min and then held at 300 °C for 1 min. The ion source and mass spectrometer quadrupole temperatures were held at 230 and 150 °C, respectively. The mass selective detector was operated in the electron ionization (EI) mode with the EI energy of 70 eV. Selected ion monitor mode was employed and the ion m/z 327 was chosen as the quantitative ion for the determination of melamine derivative.

A 100 μg/mL stock solution was prepared by dissolving 10 mg of melamine in 100 mL of methanol−water (4:1). The stock solution was then diluted with methanol to provide standard solutions of 0.05, 0.20, 1.0, 2.0, 50, 10.0, and 25.0 μg/mL, respectively. One hundred microliters of the standard solution was added into a glass vial, dried by nitrogen gas in a water bath at 50 °C, and then derivatized and detected as described above. The calibration curve was constructed by plotting the peak areas against the concentrations of standard solution and was used to determine the concentration of melamine in all subsequent analysis.

Table 1. Recoveries and CVs of Melamine Obtained from Fortified Egg Homogenates

	intraday (n = 6)		interday (n = 18)[a]	
spiked level (mg/kg)	recovery (%)	CV (%)	recovery (%)	CV (%)
0.05	95.1	5.8	95.7	6.5
0.5	94.7	4.5	96.1	5.1
2	90.2	3.7	92.4	6.3
10	89.3	5.2	90.5	4.8

[a] 6 samples each day for 3 days.

Recovery Study. The egg samples used in the recovery study were obtained from laying hens which had not been exposed to melamine. Fortified samples were prepared by adding appropriate amounts of standard solution to the control homogenized samples and were allowed to stand at room temperature for at least 30 min before extraction. For the recovery study, spiked concentrations of 0.05, 0.5, 2.0, and 10 mg/kg melamine were prepared. For each spiked level, six replicates were analyzed for each assay and three assays were repeated for three consecutive days.

RESULTS AND DISCUSSION

Method Development. Numerous methods for the detection of melamine in various biological matrixes have been reported in the literature including enzyme-linked immunosorbent assay (*8*), nuclear magnetic resonance (*9*), infrared spectroscopy (*10*), capillary zone electrophoresis (*11*), high performance liquid chromatography (*12*), liquid chromatography−tandem mass spectrometry (LC−MS/MS (*6*, *13*−*16*)) and gas chromatography−mass spectrometry (*17*−*21*). Generally, enzyme-linked immunosorbent assay, nuclear magnetic resonance and infrared spectroscopy have been used as screening methods while capillary zone electrophoresis and high performance liquid chromatography could provide quantitative results but lack qualitative ability. LC−MS/MS and GC−MS have been the most frequently used approaches for the qualitative and quantitative determination of melamine residues. In previous studies, the two methods have been used to determine melamine in fish (*6*, *11*), dairy products (*11*, *18*, *19*), soil (*17*), animal tissues (*20*) and egg (*21*). Based on the literature, a GC−MS approach to the detection of melamine in egg samples was developed for use in the current study.

Melamine is a polar chemical with three amine groups in its structure (*1*). It can be easily extracted from a variety of matrixes using methanol, acetonitrile or acid aqueous solutions. In the present study, trichloroacetic acid was used as the extraction solution. Trichloroacetic acid precipitates egg protein allowing for the preparation of clean extracts. In addition, the amino groups of melamine could be protonized in trichloracetic acid solution and thus could be easily retained on a cation exchange column for further purification. The extraction efficiency of three concentrations of trichloroacetic acid solution (2, 5 and 10%) was compared. The corresponding recoveries were 91.7, 92.7 and 84.6%, respectively (n = 3). The lowest recovery was obtained for the 10% TCA extraction, which may be attributed to a lower level of melamine being retained on the cation exchange column. Since higher concentrations of TCA did not provide additional benefits for the retention of melamine on the SPE column, a 2% solution of TCA was chosen and ultimately used as the extraction solution.

Yokley et al. reported a GC−MS method without derivatization (*17*). However, preliminary experiment conducted in our lab found this method would result in peak tailing and relatively low sensitivity, thus a silanization derivatization followed by GC−MS analysis were employed in this study. In contrast to

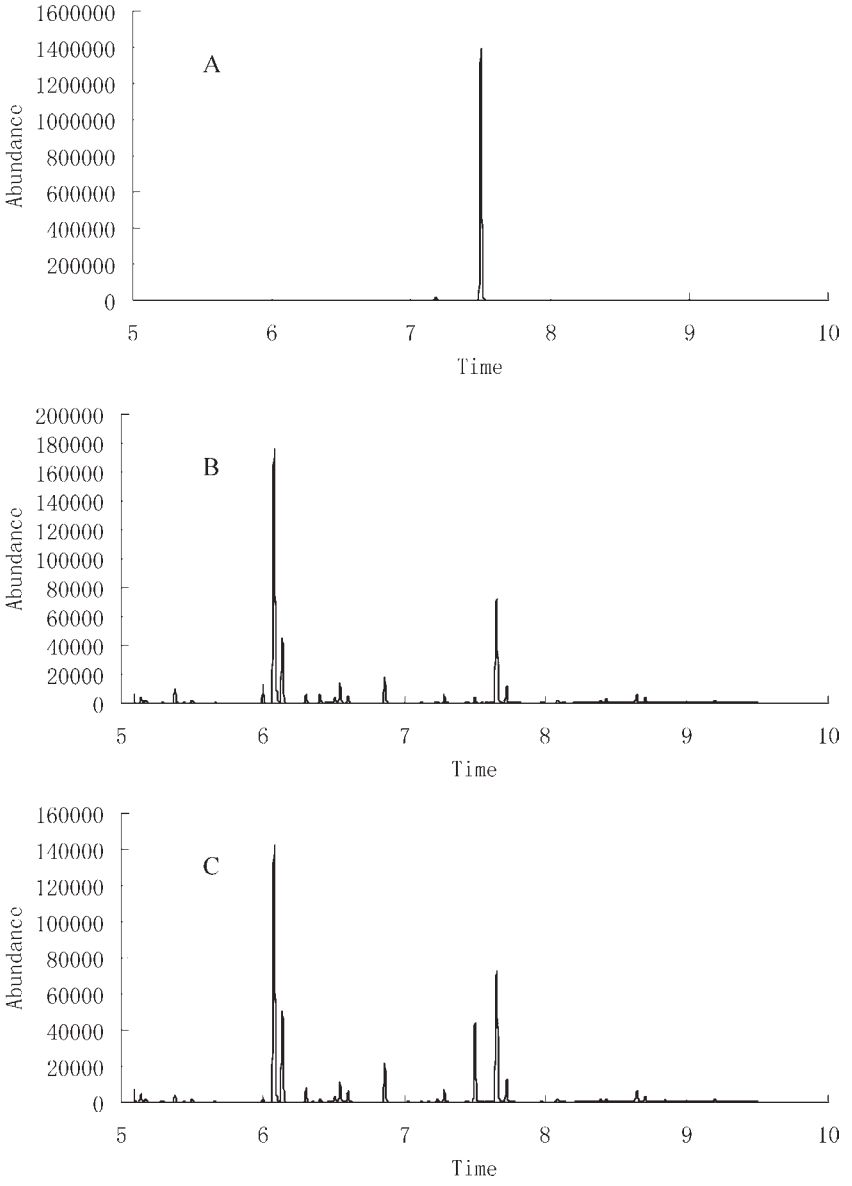

Figure 1. Typical melamine derivative chromatograms of standard solution (1 μg/mL, **A**), control sample (**B**) and spiked sample (0.05 mg/kg, **C**).

previously developed methods (*20*, *21*), acetonitrile instead of pyridine was used in the reaction medium to derivatize with melamine since pyridine is relatively toxic and has an unpleasant odor. The derivatization effect of a acetonitrile medium was generally comparable with that in pyrindine, but the derivatization product would separate to two layers when the room temperature was below 20 °C and the target derivatives did not equally distribute in the two layers. Therefore, it is important that the derivatives be kept at temperature higher than 20 °C. In the later stages of this study, we found that the addition of 50 μL of acetone to the derivatives prevented stratification.

Method Validation. The optimized method described above was validated with regard to linearity, limits of detection and quantification, accuracy and precision. The calibration curve was constructed by plotting peak areas against the concentrations of melamine and was used to determine the concentration of melamine in all subsequent analysis. The linear range was determined to be between 0.05 and 25 μg/mL with an R^2 of 0.9998. For the detection of melamine in egg samples, the limits of detection and limits of quantification, which were defined as a signal-to-noise ratio of 3:1 and 10:1 respectively, were determined to be 0.015 and 0.05 mg/kg, respectively. The average recovery of blank tissues, spiked at the limit of quantification level, were all above 90% with CVs less than 10%. The accuracy and precision of the method was evaluated by measuring recovery and the coefficient of variability (CV). The recoveries of melamine from spiked egg homogenates are summarized in **Table 1**. The recoveries at fortification levels in the 0.05 to 10 mg/kg range varied from 89.3 to 96.1% with intraday CVs of 3.7 to 5.8% and interday CVs of 4.8 to 6.5%. Typical melamine derivative chromatograms of standard solution, control sample and spiked sample are presented in **Figure 1**. The lack of interference for the melamine peak in the chromatogram suggests a high specificity of the GC−MS method and good selectivity for the sample cleanup. The above validation results demonstrate that the method developed herein satisfied all validation criteria required for quantification determination and it could therefore be used to determine melamine residues in eggs.

Application in Field Samples. The validated gas chromatograph−mass spectrometry method was then applied to determine melamine concentrations in eggs from laying hens exposed to melamine contaminated feed. For each batch of samples, a 1 μg/mL standard solution was analyzed in triplicate before and after the sample extracts to calibrate the standard curve. Also, two quality

Table 2. Melamine Concentrations (Mean ± SD) in Eggs from Laying Hens Exposed to Melamine Contaminated Feed

	contamination level of melamine in feeds				
exposure day	0 mg/kg	5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
1	ND[a]	0.12 ± 0.04[b]	0.32 ± 0.09	0.62 ± 0.09	1.42 ± 0.39
3	ND	0.11 ± 0.03	0.55 ± 0.11	0.94 ± 0.19	1.93 ± 0.22
5	ND	0.21 ± 0.06	0.57 ± 0.08	0.93 ± 0.13	1.87 ± 0.28
7	ND	0.25 ± 0.08	0.60 ± 0.12	0.93 ± 0.20	1.59 ± 0.09
9	ND	0.13 ± 0.02	0.4 ± 0.02	0.65 ± 0.10	1.07 ± 0.37
11	ND	0.14 ± 0.02	0.38 ± 0.07	0.86 ± 0.23	1.28 ± 0.23
13	ND	0.16 ± 0.06	0.37 ± 0.06	0.83 ± 0.15	1.09 ± 0.15
15	ND	0.12 ± 0.03	0.55 ± 0.12	0.95 ± 0.27	1.61 ± 0.26
means		0.16 ± 0.05	0.47 ± 0.11	0.84 ± 0.13	1.48 ± 0.33
P value		>0.05	>0.05	>0.05	>0.05

[a] ND, not detected. [b] Expressed as melamine concentration (mg/kg) ± SD ($n = 6$).

control samples with fortified melamine levels of 0.5 mg/kg were extracted and detected in each batch of samples run. This concentration was selected because it was close to that of real samples. In all cases, the recoveries for the quality control samples were in the range of 89 to 103%. The detection results are shown in **Table 2**.

Residue Deposition Study. The carryover of melamine from animal feed to animal-derived products is an important subject for risk assessment purposes. A study conducted by Andersen et al. (*6*) indicated that fish dosed with 299−471 mg/kg BW/day of melamine for 3 days contained melamine levels ranging from 81 to 210 mg/kg in catfish, from 34 to 80 mg/kg in trout, from 0.02 to 177 mg/kg in tilapia and from 58 to 94 mg/kg in salmon. In the study of Bermudez et al. (*7*), broiler chickens were fed feed containing 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% melamine from 1 to 21 days of age. For the 3.0% dose group, melamine concentrations in pectoral muscle, liver and kidney were found to be 600, 700, and 1000 mg/kg, respectively.

In the present study, relatively low levels of melamine were fed in order to investigate the safety threshold of melamine in feed. At day 1, the melamine concentrations in eggs were 0.00 (below the limit of detection), 0.12, 0.32, 0.62, and 1.42 mg/kg for the 0, 5, 25, 50, and 100 mg/kg treatment groups, respectively, which indicates that there was rapid deposition of melamine in eggs.

During the entire 15-day experiment, the average melamine concentrations in eggs were 0.00 (below limit of detection), 0.16, 0.47, 0.84, and 1.48 mg/kg for the 0, 5, 25, 50, and 100 mg/kg treatment groups, respectively, which demonstrated an apparent dose−response relationship. However, none of the melamine concentrations exceeded the maximum permitted residue level (2.5 mg/kg in food) set by the United States Food and Drug Administration (*5*), which implies that melamine contamination levels below 100 mg/kg in feed would not result in potential hazards for food safety. An additional point of interest is that, for each treatment group, the melamine levels in the eggs from days 1−15 have no significant difference ($P > 0.05$), suggesting that laying hens did not accumulate melamine for later deposition in eggs.

The relationship between the melamine concentrations in eggs and the contamination level in feeds was investigated. The melamine concentration in egg increased linearly as the contamination level in the feed increased ($P < 0.01$, **Figure 2**). The linear equation was $Y = 0.08491 + 0.01473X$ with an R^2 of 0.99885. According to this equation, laying hens fed with feed containing 164 mg/kg of melamine might well result in melamine residue concentrations exceeding the maximum permitted residue level of 2.5 mg/kg in eggs.

In conclusion, a GC−MS method was developed for the determination of melamine in eggs. This method was then employed to investigate the extent of melamine deposition in eggs obtained from laying hens fed with melamine contaminated feed. The results obtained clearly indicate the deposition characteristics of melamine in eggs, which can provide a scientific basis for the risk assessment of melamine contamination in poultry feeds fed to laying hens.

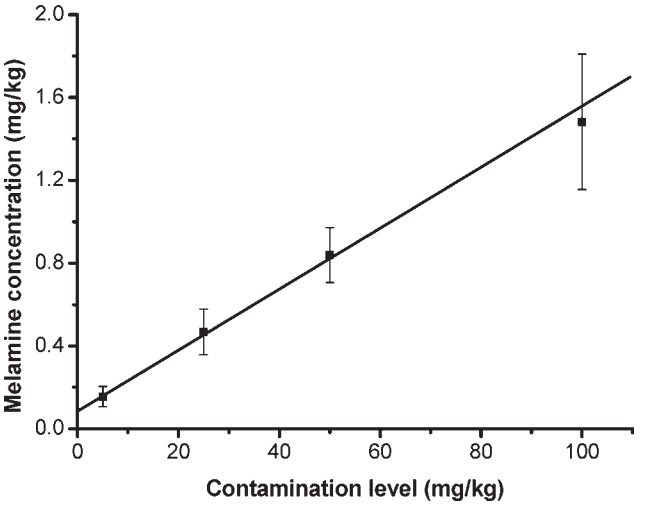

Figure 2. The relationship of melamine concentrations in eggs (*y*-axis) and contamination levels in laying hen feeds (*x*-axis).

LITERATURE CITED

(1) World Health Organization, *Background Paper on Occurrence of Melamine in Foods and Feed*. http://www.who.int/foodsafety/fs_management/Melamine_3.pdf. Accessed on Oct 15, **2009**.

(2) Organization for Economic Co-operation and Development. OECD Sudden Infant Death Syndrome Analysis United Nations Environmental Programme Publication No. 83-2501, **2002**.

(3) Shelby, M. D.; Erexson, G. L.; Hook, G. J.; Tice, R. R. Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. *Environ. Mol. Mutagen.* **1993**, *21*, 160–179.

(4) Baynes, R. E.; Smith, G.; Mason, S. E.; Barrett, E.; Barlow, B. M.; Riviere, J. E. Pharmacokinetics of melamine in pigs following intravenous administration. *Food Chem. Toxicol.* **2008**, *46*, 1196–1200.

(5) Food Drug Administration (FDA), *Update: Interim Safety and Risk Assessment of Melamine and its Analogues in Food for Humans*, http://www.fda.gov/Food/Food Safety/FoodContaminantsAdulteration/ChemicalContaminants/Melamine/ucm164520.htm. Accessed on Jan 14, **2010**.

(6) Andersen, W. C.; Turnipseed, S. B.; Karbiwnyk, C. M.; Clark, S. B.; Madson, M. R.; Gieseker, C. M.; Miller, R. A.; Rummel, N. G.; Reimschuessel, R. Determination and confirmation of melamine residues in catfish, trout, tilapia, salmon, and shrimp by liquid chromatography with tandem mass spectrometry. *J. Agric. Food Chem.* **2008**, *56*, 4340–4347.

(7) Bermudez, A. Pathology and mortality associated with graded levels of melamine fed to young broiler chickens. *Proc. Annu. Meet.—Am. Assoc. Vet. Lab. Diagn.* **2008**, No. October, 112.

(8) Garber, E. A. Detection of melamine using commercial Enzyme-Linked Immunosorbent Assay technology. *J. Food Prot.* **2008**, *71*, 590–594.

(9) Lachenmeier, D. W.; Humpfer, E.; Fang, F.; Schutz, B.; Dvortsak, P.; Sproll, C.; Spraul, M. NMR-Spectroscopy for non-targeted screening and simultaneous quantification of health-relevant compounds in foods: The example of melamine. *J. Agric. Food Chem.* **2009**, *57*, 7194–7199.

(10) Mauer, L. J.; Chernyshova, A. A.; Hiatt, A.; Deering, A.; Davis, R. Melamine detection in infant formula powder using near- and mid-infrared spectroscopy. *J. Agric. Food Chem.* **2009**, *57*, 3974–3980.

(11) Yan, N.; Zhou, L.; Zhu, Z.; Chen, X. Determination of melamine in dairy products, fish feed, and fish by capillary zone electrophoresis with diode array detection. *J. Agric. Food Chem.* **2009**, *57*, 807–811.
(12) Ono, S.; Funato, T.; Inoue, Y.; Munechika, T.; Yoshimura, T.; Morita, H.; Rengakuji, S. I.; Shimasaki, C. Determination of melamine derivatives, melame, melamineeme, ammeline and ammelide by high-performance cation-exchange chromatography. *J. Chromatogr. A* **1998**, *815*, 197–204.
(13) Sancho, J. V.; Ibanez, M.; Grimalt, S.; Pozo, O. J.; Hernandez, F. Residue determination of cyromazine and its metabolite melamine in chard samples by ion-pair liquid chromatography coupled to electrospray tandem mass spectrometry. *Anal. Chim. Acta* **2005**, *530*, 237–243.
(14) Filigenzi, M. S.; Puschner, B.; Aston, L. S.; Poppenga, R. H. Diagnostic determination of melamine and related compounds in kidney tissue by liquid chromatography/tandem mass spectrometry. *J. Agric. Food Chem.* **2008**, *56*, 7593–7599.
(15) Wu, Y. T.; Huang, C. M.; Lin, C. C.; Ho, W. A.; Lin, L. C.; Chiu, T. F.; Tarng, D. C.; Lin, C. H.; Tsai, T. H. Determination of melamine in rat plasma, liver, kidney, spleen, bladder and brain by liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* **2009**, *1216*, 7595–7601.
(16) Ibanez, M.; Sancho, J. V.; Hernandez, F. Determination of melamine in milk-based products and other food and beverage products by ion-pair liquid chromatography-tandem mass spectrometry. *Anal. Chim. Acta* **2009**, *649*, 91–97.
(17) Yokley, R. A.; Mayer, L. C.; Rezaaiyan, R.; Manuli, M. E.; Cheung, M. W. Analytical method for the determination of cyromazine and melamine residues in soil using LC-UV and GC-MSD. *J. Agric. Food Chem.* **2000**, *48*, 3352–3358.
(18) Xu, X. M.; Pen, Y. P.; Zhu, Y.; Cai, Z. X.; Han, J. L.; Huang, B. F.; Zhu, Y. Direct determination of melamine in dairy products by gas chromatography/mass spectrometry with coupled column separation. *Anal. Chim. Acta* **2009**, *650*, 39–43.
(19) Li, J.; Qi, H. Y.; Shi, Y. P. Determination of melamine residues in milk products by zirconia hollow fiber sorptive microextraction and gas chromatography-mass spectrometry. *J. Chromatogr. A* **2009**, *1216*, 5467–5471.
(20) Zhu, X. Z.; Wang, S. H.; Liu, Q.; Xu, Q.; Xu, S.; Chen, H. Determination of residues of cyromazine and its metabolite, melamine, in animal-derived food by gas chromatography-mass spectrometry with derivatization. *J. Agric. Food Chem.* **2009**, *57*, 11075–11080.
(21) Xia, X.; Ding, S. Y.; Li, X. W.; Gong, X.; Zhang, S. X.; Jiang, H. Y.; Li, J. C.; Shen, J. Z. Validation of a confirmatory method for the determination of melamine in egg by gas chromatography-mass spectrometry and ultra-performance liquid chromatography-tandem mass spectrometry. *Anal. Chim. Acta* **2009**, *651*, 196–200.

Received for review November 30, 2009. Revised manuscript received January 21, 2010. Accepted February 1, 2010. This work is supported by National Special Science Foundation for Postdoctoral Scientists of China (NO. 200902152) and Science and Technology Department of China for Science and Technology Support Program (No. 2006BAD12B03).