Environmental Pollution 238 (2018) 248–254

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Melamine and its derivatives in dog and cat urine: An exposure assessment study☆

Rajendiran Karthikraj [a], Rasya Bollapragada [a], Kurunthachalam Kannan [a, b, c, *]

[a] Wadsworth Center, New York State Department of Health, Empire State Plaza, P.O. Box 509, Albany, NY, 12201-0509, United States
[b] Department of Environmental Health Sciences, School of Public Health, State University of New York at Albany, Albany, NY, 12201-0509, United States
[c] Biochemistry Department, Faculty of Science and Experimental Biochemistry Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, 21589, Saudi Arabia

ARTICLE INFO

Article history:
Received 22 January 2018
Received in revised form
26 February 2018
Accepted 28 February 2018

Keywords:
Melamine
Cyanuric acid
Dog urine
Cat urine
Pet exposure

ABSTRACT

Melamine is a nitrogen-containing organic compound that is used in a wide range of products, including paints, plastics, and paper, as a flame retardant. A few studies have reported the occurrence of melamine and its derivatives in pet food, following a number of deaths of cats and dogs from kidney failure in 2007, which was attributed to melamine contamination in ingredients used in pet food. Nevertheless, studies that report the occurrence of melamine and its derivatives in pet urine are scarce. In this study, we measured melamine and its derivatives (i.e., ammeline, ammelide, and cyanuric acid) in dog ($n = 30$) and cat ($n = 30$) urine collected from Albany, New York, USA, during March through July 2017. The mean (±SD) concentrations of melamine, ammeline, ammelide, and cyanuric acid in dog urine were 21.1 ± 51.2, 2.3 ± 3.8, 9.9 ± 1 0.4, and 79.0 ± 105 ng/mL, respectively; the corresponding concentrations in cats were 21.4 ± 26.1, 1.2 ± 2.5, 6.1 ± 3.9, and 105 ± 94.6 ng/mL, respectively. No significant difference was observed in urinary concentrations of melamine derivatives between cats and dogs. Age and gender were important determinants of the concentrations of the target chemicals in cats and dogs. Cumulative daily intake of melamine and its derivatives was calculated on the basis of urinary concentrations and was found to be 10–500-fold below the tolerable daily intake.

1. Introduction

Pets share a living environment with humans and are exposed to a wide range of environmental toxicants present in the indoor environment (Ali et al., 2013; Dirtu et al., 2013; Severe et al., 2015). Melamine is one such chemical that has been found as an adulterant in milk products (especially infant formula) and pet foods (Baynes and Riviere, 2010; Lu et al., 2009; Tyan et al., 2009; Araujo et al., 2012). In 2007, melamine adulteration in pet foods resulted in the death of hundreds of cats and dogs in the USA (Baynes and Riviere, 2010; Jia and Jukes, 2013). Since then, attention has been paid to monitoring exposure to melamine in pets (Rovina and Siddiquee, 2015; Spink et al., 2017). Melamine (melamine resins: a combination of melamine and formaldehyde) is also widely used in lamination products (e.g., dinnerware, paints, flooring), thermosetting plastics, cleaning agents, automotive surface coatings, textile products, and moldings (Lu et al., 2009; Rovina and Siddiquee, 2015; Sugita et al., 1990; Zhang et al., 2010).

Although melamine is not directly used as a pesticide, degradation of triazine herbicides (e.g., cyromazine) can yield melamine (Roberts et al., 1999). Both melamine and cyanuric acid are by-products of coal processing and are used as adulterants in fertilizers (Field and Field, 2010; World Health Organization [WHO], 2008). Cyanuric acid is widely used as a disinfectant stabilizer in swimming pools and can be formed as a degradation product of bacterial metabolism of triazine herbicides (Huthmacher and Most, 2000; Prosen and Zupancic-Kralj, 2005). The recent production estimates for melamine and cyanuric acid are above 2 million tons annually (European Food Safety Authority [EFSA], 2010). Ammeline and ammelide are the intermediates formed during the metabolism of melamine or are formed as by-products during melamine production (Braekevelt et al., 2011; Rovina and Siddiquee, 2015).

Cats and dogs are sentinels of the human health effects of chemical exposures. Several studies have documented the

☆ This paper has been recommended for acceptance by Dr. Chen Da.

* Corresponding author. Wadsworth Center, Empire State Plaza, P.O. Box 509, Albany, NY 12201-0509, United States.
E-mail address: Kurunthachalam.Kannan@health.ny.gov (K. Kannan).

https://doi.org/10.1016/j.envpol.2018.02.089
0269-7491/

association between chemical exposure and pet health (Engdahl et al., 2017; Koestel et al., 2017; Mizukawa et al., 2016). For instance, in Minamata Bay in Japan during the 1950s, neurobehavioral symptoms were first observed in cats that consumed mercury-contaminated fish (Tsuchiya, 1992). Severe et al. (2015) suggested that pet dogs are sentinels for breast cancer in humans from exposure to persistent organic pollutants. Dirtu et al. (2013) showed relationship between concentrations of organohalogen contaminants and diabetes in cats.

Following the 2007 poisoning of cats and dogs in the USA due to melamine and cyanuric acid contamination in pet foods, the Human and Pet Food Safety Act was passed (Burns, 2007; Cocchi et al., 2010; Tran et al., 2010; Tyan et al., 2009; WHO, 2008). Although melamine is not added deliberately to pet foods, exposure to this class of chemicals through several products occurs. Thus, the European Union (EU) established a specific migration limit for melamine from plastics to foods at 30 mg/kg (Bradley et al., 2005).

Concurrent exposure to both melamine and cyanuric acid stimulates urinary stone formation (via formation of melamine-cyanurate complex) in the kidneys, which eventually leads to renal failure and death (Dobson et al., 2008; Puschner et al., 2007). Recent human studies demonstrated an association between exposure to melamine and renal injury/damage (Liu et al., 2017; Wu et al., 2018). Chronic kidney disease (CKD) is a common cause of pet illness and mortality, and the incidence of CKD in cats has been increasing for the past 10 years in the USA (Finch et al., 2016; O'Neill et al., 2015). The prevalence of feline CKD increases with age, and 30–50% of cats of ages >15 years suffer from CKD ((http://todaysveterinarypractice.navc.com/feline-chronic-kidney-disease) Bartlett et al., 2010). The prevalence of CKD in dogs has been reported at 5.8% and is manifested primarily as a geriatric disease (Bartlett et al., 2010). Despite the significance of melamine exposure in terms of the kidney health of pets, little is known about the current status of such exposure. To the best of our knowledge, studies that measure melamine, ammeline, ammelide, and cyanuric acid in cat and dog urine are not available (Cocchi et al., 2010). The present study was aimed at determining the occurrence of melamine, ammeline, ammelide, and cyanuric acid in dog and cat urine from the USA to delineate current exposure doses and potential health risks from exposure.

2. Materials and methods

2.1. Chemicals and reagents

Analytical standards of melamine (purity: 99%), ammeline (purity: 97.9%), ammelide (purity: ≥98%), and cyanuric acid (purity: 98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The isotopically-labelled internal standards (IS), melamine (ring $^{13}C_3$, 99%, amino-$^{15}N_3$, 98%), ammeline (ring $^{13}C_3$, 99%), ammelide (ring $^{13}C_3$, 99%), and cyanuric acid (ring $^{13}C_3$, 99%, amino-$^{15}N_3$, 98%), were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Commercial urine (Surine™ Negative Urine Control) was purchased from Cerilliant (Round Rock, TX, USA). Hydrochloric acid, formic acid, sodium hydroxide, and ammonium hydroxide solution were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade solvents were purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA). Methanol (optima LC/MS grade) was purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.2. Sample collection and preparation

Dog and cat urine samples were collected from three different sources in Albany, New York, USA, during March through July 2017. The samples were from a veterinary hospital (3 dogs and 5 cats), an animal shelter (10 dogs and 23 cats), and individual pet owners (17 dogs and 2 cats). Urine samples were collected using polypropylene (PP) containers provided by the laboratory. To avoid any possible contamination during sample collection, canine urines were collected directly in PP containers and feline urines were collected by cystocentesis or directly in PP containers. Details with regard to age, gender, and breed are listed in Tables S1 and S2. The urine samples were stored at −20 °C until analysis. Commercial pet foods (n = 12) were purchased from local stores in Albany, New York, USA, during March through April 2017 and were analyzed in May 2017. The target chemicals in pet foods were extracted by the method described earlier (Braekevelt et al., 2011).

Melamine, ammeline, ammelide, and cyanuric acid were determined in urine by following the methods described elsewhere, with slight modifications (Braekevelt et al., 2011; Panuwet et al., 2010). Mixed-mode solid-phase extraction method was used for the extraction of all target chemicals. A MCX cartridge was used for the extraction of melamine and ammeline, and a MAX cartridge was used for the extraction of ammelide and cyanuric acid. The detailed extraction protocol is described in the supplementary information.

2.3. Instrumental analysis

An Agilent 1260 HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA) coupled with an ABSCIEX 4500 Q-Trap mass spectrometer (Applied Biosystems, AB Sciex, Framingham, MA, USA) was used for the separation and quantification of the target chemicals. Target chemicals were separated using an Acclaim Trinity P1 column (100 mm × 2.1 mm, and 3 μm particle size; Thermo Scientific, Waltham, MA, USA) serially connected to a Betasil C18 guard column (20 mm × 2.1 mm, 5 μm particle size; Thermo Scientific, Waltham, MA, USA) with methanol (A) and ammonium acetate buffer (B) (5 mM, adjusted to pH 5.5 using acetic acid) as mobile phases (Tran et al., 2010). Mobile phase flow rate and the sample injection volumes were 250 μL/min and 20 μL, respectively. The mobile phase gradient used for the separation of target chemicals was, 10% B for 3 min initially, ramped to 65% B at 5th min which was maintained until 12th min, and then returned to the initial condition (10% B) to equilibrate for 6 min. The optimized MRM transitions and ionization modes for all target analytes are listed in Table S3. Creatinine was measured for all pet urine samples by dilution with ultra-pure water (160-fold) and injection into an HPLC-MS/MS (Xue et al., 2015). Specific gravity (SG) of urine samples was measured using a refractometer (ATAGO, Tokyo, Japan). Both creatinine and SG values of pet urine samples are listed in Tables S1 and S2.

2.4. Quality assurance and quality control

A procedural blank, spiked water, matrix blank, and two levels of matrix spike (using commercial urine) were analyzed with every 15 samples. Specimen collection tubes/containers were tested for background levels of contamination. None of the target chemicals was present in the containers. Instrument calibration curves were prepared by injecting standards at concentrations that ranged from 0.1 to 200 ng/mL, and the correlation coefficients (r^2) were >0.99. For every 10 samples, a solvent blank (methanol) and a midpoint calibration standard were injected to check for carryover and drift in instrument sensitivity. Melamine (~0.4 ng/mL) and cyanuric acid (4.36 ng/mL) were detected in procedural blanks, and these concentrations were subtracted from all reported sample concentrations. The recoveries of target chemicals spiked at concentrations of 20 and 40 ng in commercial urine and water were in the ranges of 88.0 ± 15.4% to 104 ± 1.8% (Table S3). The method limit of detection