Pharmacokinetics of Melamine and Cyanuric Acid and Their Combinations in F344 Rats

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The intentional adulteration of pet food with melamine and cyanuric acid has been implicated in the kidney failure and death of a large number of cats and dogs in the United States. Although individually these compounds present low toxicity in a range of experimental animals, coexposure can lead to the formation of melamine cyanurate crystals in the nephrons and eventual kidney failure. Given this mode of action, a good understanding of the pharmacokinetic profiles of melamine and cyanuric acid and their combinations is essential to define properly the risk associated with different exposure scenarios. Previous studies have investigated the individual pharmacokinetic profiles of melamine and cyanuric acid. In this work, we report a comparison between the pharmacokinetic profiles of melamine and cyanuric acid administered individually, administered simultaneously as the individual compounds, and administered as a preformed melamine cyanurate complex. Although the oral coadministration of 1 mg/kg body weight of melamine and cyanuric acid did not alter significantly the pharmacokinetic profiles in relation to those determined upon individual oral administration of each compound, the administration of equal amounts of each triazine as the preformed melamine cyanurate complex significantly altered the pharmacokinetics, with reduced bioavailability of both compounds, lower observed maximum serum concentrations, delayed peak concentrations, and prolonged elimination half lives. These results indicate that in order to estimate properly the combined nephrotoxic potential of melamine and cyanuric acid, the experimental design of toxicological experiments and the evaluation of animal or human exposure scenarios should consider the detailed mode of exposure, with particular emphasis on any possible ex vivo formation of melamine cyanurate.

Key Words: melamine; cyanuric acid; melamine cyanurate; pharmacokinetics.

MATERIALS AND METHODS

Chemicals. Melamine (Aldrich, stated purity 99%, CAS 108-78-1) and cyanuric acid (Fluka, anhydrous, stated purity > 98%, CAS 108-80-5) were obtained from Sigma-Aldrich (St Louis, MO). Carboxymethylcellulose (CMC,
sodium salt, high viscosity) was obtained from MP Biomedicals (Solon, OH). Melamine cyanurate complex (CAS 37640-57-6) was prepared by coprecipitation of aqueous equimolar solutions of melamine and cyanuric acid. The precipitate thus obtained was filtered, thoroughly washed with hot water (80–90°C), and dried under reduced pressure. The purity and identity of the test articles were confirmed by high-performance liquid chromatography (HPLC) coupled with ultraviolet detection and electrospray mass spectrometry and by gas chromatography-mass spectrometry in electron impact mode. All solvents used for the dosing solution analyses were purchased from Sigma-Aldrich and met or exceeded American Chemical Society specifications.

Preparation and certification of the dosing solutions. Intravenous (iv) dosing solutions were prepared by dissolving melamine or cyanuric acid at the concentration of 1 mg/ml in normal saline and filter sterilizing the solutions. The gavage solutions were prepared by dissolving the respective compound in a 0.1% CMC solution in water. These solutions were prepared at the following concentrations: individual gavage studies with melamine or cyanuric acid—200 µg/ml; combined gavage studies with melamine and cyanuric acid—400 µg/ml each of melamine or cyanuric acid. The gavage suspension of melamine cyanurate was prepared by adding finely pulverized melamine cyanurate at a concentration of 400 µg/ml to 0.1% CMC in water. All dosing formulations were analyzed with a Thermo Surveyor Plus HPLC (Thermo, Waldham, MA) using a Synergi Polar RP 80A, 4 µm, 2 × 250 mm column (Phenomenex, Torrance, CA) eluted isocratically at 400 µl/min with 95% 10mM ammonium phosphate and 5% acetonitrile. The eluate was monitored at 209 nm. All dosing solutions were determined to be between 99.6 and 104.9% of their nominal concentrations. The melamine cyanurate suspension was determined to be at 102.3 ± 1.4% of its nominal concentration, as determined by analysis of three independent samples drawn under simulated dosing conditions.

Animals, dosing procedure, and blood collection schedule. All procedures involving the care and handling of animals were reviewed and approved by the Institutional Animal Care and Use Committee at the National Center for Toxicological Research (NCTR). F344 rats were obtained from the breeding colony at the NCTR at 6 weeks of age. The rats were weight-ranked (acceptable weight: ± 20% of the mean body weight [bw]), and randomly assigned to treatment groups (eight males and eight females per iv dose group; six males and six females per gavage dose group) that were fed ad libitum with NIH-31 irradiated feed. The animals were housed in individual polycarbonate cages with hardwood chip bedding and the room environmental controls were set to maintain a relative humidity of 40–70% and a 12-h light/day cycle. At 10 weeks of age, the animals were injected in the saphenous vein with 1 ml/kg bw of 1 mg/ml solution of melamine or cyanuric acid in saline (iv treatment groups), were gavaged with 5 ml/kg bw of a 200 µg/ml solution of melamine or cyanuric acid in 0.1% CMC (individual gavage groups), were gavaged with 2.5 ml/kg of a 400 µg/ml melamine solution in 0.1% CMC followed, within less than 1 min, by a separate gavage with 2.5 ml/kg of a 400 µg/ml cyanuric acid solution in 0.1% CMC (combined melamine and cyanuric acid gavage group), or were gavaged with 5 ml/kg bw of a 400 µg/ml suspension of melamine cyanurate in 0.1% CMC in water. The iv injections were performed with zero-dead volume disposable syringes equipped with 28 gauge½ inch needles (Terumo Medical Corporation, Somerset, NJ), and the gavages were conducted with syringes equipped with ball-tipped stainless steel gavage needles. Approximately 100-µl samples of blood were collected from the tail veins with zero-dead volume 1 cc disposable syringes with permanently attached 25 gauge 5/8 inch needles (Terumo Medical Corporation, Somerset, NJ) at 0 (pretreatment), 5, 15, and 30 min, and 1, 2, 3, 4, 5, and 6 h after treatment for the iv treatment groups or 0 (pretreatment), 0.5, 1, 2, 3, 4, 5, and 6 h for the gavage treatment groups. At 8-h post treatment, the animals were euthanized by deep anesthesia with carbon dioxide and terminal exsanguination, providing an 8-h blood sample. The blood samples were allowed to clot at room temperature and the serum samples, prepared by centrifugation, were stored at −80°C.

Quantification of melamine and cyanuric acid in serum by UPLC-MS/MS. The concentrations of melamine and cyanuric acid in the serum samples were determined by isotopic dilution mass spectrometry as described in Jacob and Gamboa da Costa (2011). Briefly, the methodology incorporated 13C3-labeled cyanuric acid and 15N3-labeled melamine as internal standards and was based on ion-exchange solid-phase extraction and ultra-performance liquid chromatography coupled with electrospray tandem mass spectrometry in multiple reaction monitoring mode (UPLC-ESI-MS/MS). The method used 15 µl samples of serum and afforded lower limits of quantification for melamine and cyanuric acid of, respectively, 5 and 10 ng/ml.

Pharmacokinetic and statistical analyses. Plots of serial serum concentrations of melamine or cyanuric acid versus time after melamine, cyanuric acid, melamine and cyanuric acid, or melamine cyanurate oral or iv administration to individual rats were analyzed using model-independent pharmacokinetic analysis (PK Solutions 2.0 software, Summit Research Services, Montrose, CO). Natural log-linear plots were fit to one (elimination) or two (distribution/absorption) kinetic phases corresponding to elimination and distribution/absorption. The first-order elimination rate constants (kelim) for melamine or cyanuric acid were determined from the terminal slope of the respective curves, and the first-order distribution/absorption rate constants (kD/A) were determined after subtracting the contribution from the terminal elimination phase of the respective curves (i.e., feathering). Internal exposures to melamine or cyanuric acid were determined from area-under-the-concentration (AUC) curve using the trapezoidal rule. The Cmax, and T max after oral treatments were observed values. Half-lives (t1/2 [E]lim and t1/2[D/A]) were determined from rate constants using the relationships: half-life = ln 2/k (E)lim and ln 2/k (D/A), respectively, Cinitial after iv treatment was the extrapolated y-axis of the elimination curve. The volume of distribution (Vd) was calculated using iv administration data from the relationships: Vd = dose/(AUCiv × kelim) and Vd = dose/Cinitial. Both gave comparable values. Bioavailability (the fraction absorbed, F) was calculated as the ratio of AUCoral to AUCiv. The data are reported as the mean ± SD, with the exception of Tmax, which is reported as the median and range. The SD (σiv) for bioavailability (F) was calculated from the following relationship:

\[(σiv/Z)^2 = (σA/B)^2 + (σB/A)^2\]

where Z = A/B; A = AUCoral; B = AUCiv; (σA) = SD of AUCoral; and (σB) = SD of AUCiv.

Statistical analyses (with the exception of Tmax statistical analyses) were conducted by one-way ANOVA, with pairwise comparisons being conducted by the Student-Newman-Keuls method. Statistical analyses of Tmax values were conducted, as appropriate, by Kruskal-Wallis one-way ANOVA on Ranks, with pairwise comparisons being conducted by Dunn’s method or Mann-Whitney Rank Sum tests. p values < 0.05 were considered to be significant.

RESULTS

Intravenous Treatments

Groups of male and female F344 rats were injected in the saphenous vein with a solution of melamine or cyanuric acid in saline to afford a dose of 1 mg/kg bw. Figures 1A and 1B depict the serum concentration profiles of melamine and cyanuric acid in the male (black line) and female (gray line) rats as determined by isotopic dilution UPLC-ESI-MS/MS at various intervals after dosing. Model-independent pharmacokinetic analysis revealed clear differences between the pharmacokinetic parameters of melamine and cyanuric acid for both sexes, with cyanuric acid showing a higher initial concentration (Cinitial), a smaller AUC, a more rapid elimination (t1/2 [E]lim), and a small volume of distribution (Vd) (Table 1). In addition, intersex differences were observed in the melamine-exposed rats, with the males presenting slightly higher, but statistically significant, values...
FIG. 1. Time concentration profiles of melamine (MEL) and/or cyanuric acid (CYA) obtained upon analysis of serum samples from rats treated iv with 1 mg/kg bw of melamine alone (panel A), treated iv with 1 mg/kg bw of cyanuric acid alone (panel B), gavaged with 1 mg/kg bw of melamine alone (panel C), gavaged with 1 mg/kg bw of cyanuric acid alone (panel D), gavaged with 1 mg/kg bw of melamine and 1 mg/kg bw of cyanuric acid (panels E [melamine profile] and F [cyanuric acid profile]), or gavaged with 2 mg/kg bw of melamine cyanurate complex (panels G [melamine profile] and H [cyanuric acid profile]). The male rat traces are represented in black, and the female traces are represented in gray. The error bars represent ± SD (n = 8 per sex in the iv treatments and n = 6 per sex in the oral treatments).
for $C_{\text{initial}}$ and AUC and slightly lower values for $t_{1/2}$ (Elim) and $V_d$ than those determined in the females (Table 1).

**Oral Exposure to Melamine or Cyanuric Acid**

Male and female F344 rats were gavaged with 5 ml/kg bw of a 200 $\mu$g/ml solution of melamine or cyanuric acid to afford an exposure of, respectively, 1 mg/kg bw melamine or cyanuric acid. Figures 1C and 1D depict the concentration profiles of melamine and cyanuric acid in the male (black line) and female (gray line) rats as determined by isotopic dilution UPLC-ESI-MS/MS for up to 8 h after treatment. With the exception of $C_{\text{max}}$ for cyanuric acid and $t_{1/2}$ (Elim) for both compounds, there were no significant differences between the serum concentration profiles of male versus female rats (Table 2). There were clear differences between the profiles obtained upon oral versus iv exposure to the same doses of melamine or cyanuric acid, with the AUCoral being lower and the $t_{1/2}$ (Elim)oral being longer. This reflects the delayed absorption of these triazines in the gastrointestinal tract upon oral administration. As was observed with the iv dosing, the pharmacokinetic analysis revealed clear differences between the pharmacokinetic parameters of melamine and cyanuric acid for both sexes, with cyanuric acid showing a higher maximum concentration ($C_{\text{max}}$), a smaller AUC, and a more rapid elimination ($t_{1/2}$ (Elim)) (Table 2). The bioavailabilities of melamine and cyanuric acid determined by the ratio of the AUC obtained upon oral gavage and the AUC obtained upon iv administration of each compound ranged from 74% (cyanuric acid in male animals) to 98% (melamine in female animals) (Table 3).

**Oral Coexposure to Melamine and Cyanuric Acid**

Groups of male and female F344 rats were gavaged with 2.5 ml/kg bw of a 400 $\mu$g/ml melamine solution and immediately (< 1 min) after were gavaged with 2.5 ml/kg bw of a 400 $\mu$g/ml cyanuric acid solution to afford an oral coexposure to 1 mg/kg bw of melamine and 1 mg/kg bw of cyanuric acid. Figures 1E and 1F depicts the concentration profile of melamine and cyanuric acid in the male (black line) and female (gray line) rats as determined by isotopic dilution UPLC-ESI-MS/MS for up to 8 h after treatment. As suggested by a comparison of the concentration curves of the individual oral exposure to melamine (Fig. 1C) or cyanuric acid (Fig. 1D) and the combined exposure curves (respectively Figs. 1E and 1F), the calculated pharmacokinetic parameters were essentially unchanged by the coadministration of the triazines as compared with the compounds individually (Table 2).

**Oral Exposure to Melamine Cyanurate Complex**

Male and female F344 rats were gavaged with 5 ml/kg bw of a 400 $\mu$g/ml suspension of melamine cyanurate. Figures 1G and 1H depict the concentration profile of melamine and cyanuric acid in the male (black line) and female (gray line) rats at various intervals for up to 8 h after dosing. As suggested

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**Table 1**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Treatment</th>
<th>Melamine</th>
<th>Cyanuric acid</th>
<th>M</th>
<th>Cmax (mg/ml)</th>
<th>AUC (ng-h/ml)</th>
<th>$t_{1/2}$ (Elim) (h)</th>
<th>$V_d$ (ml/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Melamine</td>
<td>1,189 ± 77</td>
<td>2,101 ± 345</td>
<td>2,309 ± 191</td>
<td>1,925 ± 135</td>
<td>1,43 ± 0.12</td>
<td>1.31 ± 0.08</td>
<td>0.82 ± 0.06</td>
</tr>
<tr>
<td>Female</td>
<td>Melamine</td>
<td>972 ± 153</td>
<td>1,43 ± 0.12</td>
<td>1,744 ± 293</td>
<td>1,744 ± 293</td>
<td>1,43 ± 0.12</td>
<td>1.31 ± 0.08</td>
<td>0.82 ± 0.06</td>
</tr>
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</table>

Data are reported as the mean ± SD; n = 8.

Significantly different ($p < 0.001$) from the comparable measurement obtained for melamine for the same sex of rats. Significantly different ($p < 0.05$) from the comparable measurement obtained in male rats.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sex</th>
<th>Melamine, C_AUC (ng-h/ml)</th>
<th>Melamine, t_{1/2} (h)</th>
<th>Cyanuric acid, C_AUC (ng-h/ml)</th>
<th>Cyanuric acid, t_{1/2} (h)</th>
<th>Cyanuric acid, C_AUC (ng-h/ml)</th>
<th>Melamine cyanurate, C_AUC (ng-h/ml)</th>
<th>Melamine cyanurate, t_{1/2} (h)</th>
<th>Melamine cyanurate, C_AUC (ng-h/ml)</th>
<th>Melamine cyanurate, t_{1/2} (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melamine</td>
<td>Male</td>
<td>581 ± 28</td>
<td>10.0 (5.1–10.0)</td>
<td>979 ± 79</td>
<td>0.5 (0.5–1.0)</td>
<td>907 ± 132</td>
<td>1.5 (1.0–2.0)</td>
<td>919 ± 229</td>
<td>0.5 (0.5–1.0)</td>
<td>919 ± 229</td>
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<tr>
<td>Melamine</td>
<td>Female</td>
<td>615 ± 27</td>
<td>10.0 (5.1–10.0)</td>
<td>907 ± 132</td>
<td>0.5 (0.5–1.0)</td>
<td>919 ± 229</td>
<td>0.5 (0.5–1.0)</td>
<td>1.99 ± 0.99</td>
<td>1.0 (0.5–1.0)</td>
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</tr>
<tr>
<td>Melamine cyanurate</td>
<td>Male</td>
<td>135 ± 21</td>
<td>3.0 (1.0–1.5)</td>
<td>861 ± 43</td>
<td>0.5 (1.0–1.5)</td>
<td>182 ± 95</td>
<td>1.0 (1.0–1.5)</td>
<td>1375 ± 117</td>
<td>1.0 (1.0–1.5)</td>
<td>1375 ± 117</td>
</tr>
<tr>
<td>Melamine cyanurate</td>
<td>Female</td>
<td>154 ± 27</td>
<td>1.0 (1.0–1.5)</td>
<td>822 ± 95</td>
<td>0.5 (1.0–1.5)</td>
<td>1353 ± 62</td>
<td>1.0 (1.0–1.5)</td>
<td>1353 ± 62</td>
<td>1.0 (1.0–1.5)</td>
<td>1353 ± 62</td>
</tr>
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</table>

Data are reported as the mean ± SD, except for C_{max}, which is reported as the median, with the range reported in parentheses. n = 6.

**DISCUSSION**

The study of the combined toxicity of melamine and cyanuric acid constitutes a remarkable example of the complexities involved in the toxicological assessment of mixtures. Although individually melamine or cyanuric acid present low toxicities in a range of experimental animals, recent events involving the adulteration of pet food with “scrap melamine,” containing melamine and cyanuric acid, revealed that oral coexposure to these triazines can lead to acutely nephrotoxic effects (Brown et al., 2007; Dobson et al., 2008; Puschner et al., 2007; World Health Organization 2009).

Although the detailed mechanism of nephrotoxicity remains to be clarified, the data currently available demonstrate that upon coexposure to melamine and cyanuric acid, a crystalline complex of melamine cyanurate forms in the lumen of the nephrons, impairing their function and potentially leading to organ failure. The formation and accumulation of the complex are consistent with the solubility differential of melamine (ca. 3200 mg/l) and cyanuric acid (ca. 2000 mg/l) versus that of melamine cyanurate (ca. 2.2 mg/l), and it is believed that the physiological concentration gradient in the nephron loop of Henle may facilitate the nucleation of the complex and subsequent crystal growth, justifying the organ specificity of the effects (Dobson et al., 2008; Tolleson, 2008). Given this mode of action, a good understanding of the pharmacokinetic profiles of melamine and cyanuric acid and their combination is essential to define properly the risk associated with different oral exposure scenarios. A number of previous studies have addressed various aspects of the melamine pharmacokinetics in rats (Mast et al., 1983; Sugita et al., 1991; Wu et al., 2010; Yang et al., 2009), pigs (Baynes et al., 2008; Buur et al., 2008), rhesus monkey (Liu et al., 2010), goats (Baynes et al., 2010), and fish (Andersen et al., 2011; Reimschuessel et al., 2010; Xue et al., 2011). A limited set of studies have described the pharmacokinetic behavior of cyanuric acid in the rat (Hammond et al., 1986), dog (Hammond et al., 1986), and humans (Allen et al., 1982); however, only a single study has been published addressing pharmacokinetic aspects of a combined exposure to melamine and cyanuric acid, and this was
conducted in rainbow trout (Xue et al., 2011). Given the substantial anatomical and physiological differences between fish and mammalian kidneys and the fact that the only exposure studied was an oral coexposure to melamine and cyanuric acid, further studies on the combined pharmacokinetics of melamine and cyanuric acid and their combinations were warranted. In this study, we compared the pharmacokinetics of melamine and cyanuric acid as administered to F344 rats individually by iv route, individually per os, in combination per os, and as preformed melamine cyanurate complex per os. A dose of 1 mg/kg bw was selected for the individual exposures to melamine and cyanuric acid because it was expected to afford quantifiable serum levels at extended time periods using our recently developed UPLC isotopic dilution mass spectral method (Jacob and Gamboa da Costa, 2011) and was below the threshold of exposure at which we have observed significant nephrotoxic effects in subchronic studies with a combination of melamine and cyanuric acid in rats (manuscript under preparation). The absolute dose of each compound was maintained in the combination studies, in order to allow a direct comparison of the concentration-time profiles of each triazine in serum. Given the small difference between the molecular weights of melamine (126.1 g/mol) and cyanuric acid (129.1 g/mol), the doses were nearly equimolar for each triazine. As expected from previously published data, the pharmacokinetics of melamine and cyanuric acid were noticeably distinct both by iv (Figs. 1A and 1B; Table 1) or oral routes (Figs. 1C and 1D; Table 2), with melamine presenting longer elimination half lives upon iv (ca. 1.3–1.4 h) or oral dosing (ca. 1.6–1.9 h) compared with those determined for cyanuric acid upon iv (ca. 0.6–0.7 h) or oral administration (ca. 0.8–1.0 h). These values are comparable to those previously published for melanine (t1/2 ≈ 1.3–4.9 h) (Mast et al., 1983; Wu et al., 2010; Yang et al., 2009) and cyanuric acid (0.5–1 h) in rats (Hammond et al., 1986). The bioavailability of melamine in the male (F = 0.83 ± 0.08) and female rats (F = 0.98 ± 0.15) (Table 3) was also comparable with the values previously reported in the literature for rats (F ≈ 0.73–0.98) (Wu et al., 2010; Yang et al., 2009); however, to the best of our knowledge, the bioavailability determined for cyanuric acid in this study (F = 0.74 ± 0.11 for male rats; F = 0.79 ± 15 for female rats) constitutes the first report of this parameter for rats in the literature.

Given the instantaneous formation of melamine cyanurate known to occur upon mixing solutions of melamine and cyanuric acid in vitro, we anticipated that the sequential gavage of melamine and cyanuric acid in the combined oral exposure experiment might result in the coprecipitation of the triazines in the stomachs of the rats, impairing to some degree their efficient absorption. Surprisingly, the individual concentration curves for melamine (Fig. 1E) and cyanuric acid (Fig. 1F) in the combined treatment were almost indistinguishable from those resulting from the individual administration of each triazine (respectively, Figs. 1C and 1D). A detailed analysis of the main pharmacokinetic parameters (Table 2) failed to reveal any noteworthy differences between the individual and combined exposures, with bioavailabilities in the combined treatment ranging from 0.83 to 0.91 for melamine and 0.66–0.78 for cyanuric acid (Table 3). In contrast, the administration of equal amounts of each triazine as a preformed melamine cyanurate complex revealed very substantial differences in the concentration curves in both sexes for melamine (Fig. 1G) and cyanuric acid (Fig. 1H). Treatment with preformed melamine cyanurate led to significantly lower bioavailabilities for both melamine (F ≈ 0.40–0.49) and cyanuric acid (F ≈ 0.26–0.40) (Table 3) and to substantially lower observed Cmax values, a later Tmax, and prolonged elimination half lives (Table 2). We hypothesize that with the combined treatment with melamine and cyanuric acid, the hydrochloric acid naturally present in the stomach of the rats efficiently prevents the coprecipitation of the triazines as a melamine cyanurate complex by protonating the amine groups of melamine and disrupting the formation of the hydrogen bonds that stabilize the lattice of

### TABLE 3

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<tbody>
<tr>
<td>Melamine</td>
<td>0.83 ± 0.08</td>
<td>---</td>
<td>0.98 ± 0.15</td>
<td>---</td>
</tr>
<tr>
<td>Cyanuric acid</td>
<td>---</td>
<td>0.74 ± 0.11</td>
<td>---</td>
<td>0.79 ± 0.15</td>
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<td>Melamine and</td>
<td>0.83 ± 0.10</td>
<td>0.66 ± 0.10</td>
<td>0.91 ± 0.11</td>
<td>0.78 ± 0.14</td>
</tr>
<tr>
<td>cyanuric acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melamine cyanurate</td>
<td>0.40 ± 0.10&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.26 ± 0.06&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.49 ± 0.11&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.40 ± 0.10&lt;sup&gt;cd&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>a</sup>Data are presented as the mean ± SD.
<sup>b</sup>Bioavailability was calculated as the ratio of AUCoral for melamine or cyanuric acid to, respectively, the AUCiv for melamine or cyanuric acid individually.
<sup>c</sup>Significantly different (p < 0.001) from the same sex of rats treated with melamine or cyanuric acid individually.
<sup>d</sup>Significantly different (p < 0.001) from the same sex of rats treated with melamine and cyanuric acid.
melamine cyanurate; however, with the preformed melamine cyanurate treatment, the acidic environment only slowly and partially decomposed the complex to the individual triazines. The slow and partial decomposition are in agreement with the lower bioavailabilities, lower C<sub>max</sub>, delayed T<sub>max</sub>, and longer elimination half lives and is consistent with the outcome of acute toxicity studies conducted in rats gavaged with melamine cyanurate complex (Babayan and Aleksandryan, 1985). In these studies, the authors determined that the LD<sub>50</sub> for melamine cyanurate in rats was 4110 mg/kg bw, a value more than two orders of magnitude above that at which acute renal failure and lethality has been reported for a combination of melamine and cyanuric acid in rats (Jacob et al., 2011). Considering the mode of action of a combination of melamine and cyanuric acid, the threshold of toxicity is expected to be ultimately determined by the minimum coexposure capable of yielding a serum concentration and subsequent urine filtrate concentration of melamine and cyanuric acid that exceeds the value required for nucleation and initial crystal formation in the nephron. Moreover, it is probable that the subsequent crystal growth is governed by factors such as the critical size AUC attained upon exposure that is above the limit of solubility of melamine cyanurate. In light of the results presented herein, it is clear that the detailed mode of administration of melamine and cyanuric acid may have a profound impact on the nephrotoxic potential. These observations suggest that experimental study designs that allow the ex vivo formation of melamine cyanurate (e.g., gavaging with preblended melamine and cyanuric acid formulations) may underestimate the nephrotoxic potential of the mixture. Our results also highlight that a proper definition of the relative risks associated with animal or human exposure scenarios should take into consideration the detailed mode of coexposure to melamine and cyanuric acid and emphasize the importance of internal exposure measurements in toxicological studies.

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