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Amoxicillin–Clavulanic Acid and Trimethoprim–Sulfamethoxazole in Rodent Feed and Water: Effects of Compounding on Antibiotic Stability

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We assessed the concentrations of 2 antibiotic combinations, amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole when compounded in reverse osmosis [RO] (pH 6.0), tap (pH 6.7), and acidified water (pH 2.6) over 7 d, and pre- and post-pelleting, post-gamma irradiation and shipping, and monthly until 180 d post-milling in feed. Amoxicillin concentrations in RO and tap water varied between 1.18 and 1.29 mg/ml, and 1.09 and 1.22 mg/ml, respectively. The concentration of amoxicillin declined immediately and remained between 0.43 and 0.50 mg/ml in acidified water. Clavulanic acid exhibited a slow time-dependent decrease in concentration to 0.05 mg/ml at day 7 in RO water, immediately declined and varied from 0.02 to 0.05 mg/ml in tap water, and was undetectable in acidified water. Trimethoprim and sulfamethoxazole concentrations were near expected in RO, tap, and acidified water. In food, amoxicillin, trimethoprim, and sulfamethoxazole concentrations were each reduced to approximately 60% of expected after pelleting, but remained stable thereafter for 180 d. The initial clavulanic acid concentration in feed was less than 10% of expected and was undetectable after 1 mo. Plasma drug concentrations were determined in C57BL/6NCrl mice at 4 h after commencement of the dark and light cycles following administration of antibiotic food for at least 72 h. Plasma amoxicillin and sulfamethoxazole concentrations were 3- and 10-fold greater, respectively, during the dark period. Plasma levels of clavulanic acid and trimethoprim were consistent at both time points. These results indicate that the antibiotic concentration can be influenced by compounding in feed and water, and differs in plasma during the light and dark phases of the photoperiod.

Abbreviations: ESI, electrospray ionization; HPLC, high-pressure liquid chromatography; MIC, minimal inhibitory concentration; RO, reverse osmosis

'Off-label' use of human or animal medications is common in both companion animal and laboratory animal populations. Unlike treating pets, the individual dosing of many oral or injectable medications for the prescribed duration is often not feasible in laboratory rodents. Laboratory animal specialists frequently must treat large populations of rodents and have developed efficient ways of delivering oral antibiotics or other medications, including compounding medication into feed and administering medication through the drinking water.[3,5] These methods of administration are highly efficient when the medication is distributed during scheduled cage, feed, or water bottle changes. In addition, drug administration by gavage or by intravenous or intramuscular injection, may require more than momentary restraint, cause distress, or be subject to complications, such as inadvertent intrapulmonary administration, for example.

Several types of antibiotic-containing rodent feed are commercially available. In addition, various vendors compound antibiotics into rodent feed at the customer's direction. Antibiotics are available for administration in drinking water for poultry and livestock, and their effectiveness and stability has been evaluated.[30] Several antibiotics have been evaluated for off-label use in rodents; however, the number of pharmaceuticals evaluated is limited.[5,6,9] In contrast, many antibiotics or antibiotic combinations are produced for oral administration, generally as suspensions, for children and companion animals. Although the availability of these agents dramatically increases the laboratory animal specialist's armamentarium, most have not been evaluated for off-label use.

Amoxicillin, amoxicillin–clavulanate (amoxicillin trihydrate and clavulanic acid potassium salt), and trimethoprim–sulfamethoxazole are broad-spectrum antibiotics that have been used in food and water to treat laboratory rodents for *Corynebacterium*- associated hyperkeratosis or to prevent pneumocystosis.[17,31,42] The addition of antibiotics to food has been demonstrated to be efficacious in mice infected with *Helicobacter* sp. and *Pasteurella pneumotropica*.[9,10,15] Genetically modified or immunocompromised mice can receive prophylactic or therapeutic antibiotics in water or food.[31,42] Although these methods of drug administration are used routinely in vivaria, the effect of compounding the medication into either feed or water has not been evaluated critically. Moreover, limited information is available that demonstrates that the antibiotic concentrations attained in plasma reach levels expected to be efficacious for respective pathogen(s).

Compounding antibiotic into water may be influenced by the water type (for example, tap, acidified, or purified by reverse-osmosis) or the dilution used. Similarly, antibiotic stability in feed may be affected by pelleting (which subjects the ingredients to moisture, elevated temperatures, and pressure), shipping and storage, and postproduction processing such as gamma irradiation. The provision of an inadequate antibiotic dose for prolonged duration increases the risk that the animals' autoch-

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thonous flora will develop antibiotic resistance.[26,27] In addition, the compounding or off-label manipulation of medication into a secondary matrix such as food or water may compromise the chemical stability, purity, and subsequent potency of the active ingredient.[13,17,24,25,37] The American Veterinary Medical Association and Food and Drug Administration have addressed these issues.[2,35]

In the current study, we assessed the concentrations of 2 common broad-spectrum antibiotic combinations, amoxicillin–clavulanic acid and trimethoprim–sulfamethoxazole, when compounded in reverse-osmosis (RO), tap, and acidified water over a 7-d period under simulated conditions of use. We also conducted experiments to quantify antibiotic stability in antibiotic-compounded rodent feed before and after pelleting, before and after gamma irradiation and shipping, and after prolonged storage under typical vivarium storage conditions. Further, we determined the plasma antibiotic concentrations in mice during both the dark and light phase of the photoperiod after their consumption of antibiotic-compounded rodent feed combinations.

Materials and Methods

Analysis of antibiotic stability in drinking water. ***Amoxicillin–clavulanic acid.*** Three individual bottles of combined amoxicillin and clavulanic acid powders (Amoxicillin and Clavulanate Potassium for Oral Suspension, Penn Labs, Philadelphia, PA), which provide 400 and 57 mg, respectively, per 5 ml on reconstitution, were prepared with tap water as instructed by the manufacturer to create a liquid suspension for oral use.[3] Antibiotic dilutions were performed with municipal tap water (pH 7.0), acidified water (pH 2.6) obtained from a bottle filler and proportioner (model BFS-674, Edstrom Industries, Waterford, WI) providing 5-µm filtered tap water acidified with hydrochloric acid, or RO-purified water at 18 MΩ-cm resistivity and 0.05-µm ultramicrofiltered (pH 6.0; PureLab Ultra Analytical, Siemens Water Technologies, Warrendale, PA). Aliquots (6 ml) of the oral suspension were removed and diluted to 500 ml with either acidified water, tap water, or RO-purified water and used to generate 3 bottles per water type. Nine clean polysulfone water bottles (Thoren Caging Systems, Hazleton, PA) were filled with the diluted antibiotic solution (3 containing each water type) providing a final expected concentration of 0.95 mg amoxicillin and 0.135 mg clavulanic acid per milliliter. The dose was extrapolated from the twice-daily dosage of 100 mg amoxicillin per kilogram body weight for mice, assuming a C57BL6/J mouse drinks 6 ml of water daily.[32] The bottles were inverted 4 to 5 times, and 3 ml were removed from each bottle for sample analysis (0-h sample). Each bottle subsequently was placed on a wire-bar lid containing pelleted rodent diet (Purina 5058, Purina Mills International, Richmond, IN) inside a polysulfone shoebox cage (model 9, Thoren Caging Systems) containing autoclaved aspen chip bedding (PWI, St-Hyacinthe, Quebec, Canada) on an individually ventilated cage rack (Maxi-Miser Positive Individually Ventilated System, Thoren Caging Systems). Macroenvironmental conditions were maintained between 30% and 70% relative humidity and 22.2 ± 1.0 °C with 10 to 15 air changes per hour. Water bottles were inverted 4 to 5 times prior to collection of additional 3-ml samples at 48, 72, 120, and 168 h after reconstitution. Samples were frozen at –20 °C and analyzed by high-pressure liquid chromatography and electrospray ionization mass spectroscopy (HPLC ESI–MS) as described in a later section.

Trimethoprim–sulfamethoxazole. Three individual bottles of trimethoprim and sulfamethoxazole suspension (Sulfamethoxazole and Trimethoprim Oral Suspension, Hi-Tech Pharmacal, Amityville, NY), providing 200 and 40 mg, respectively, of the 2 antibiotics per 5 ml, were shaken gently and inverted 4 or 5 times.[29] A 10-ml aliquot of the oral suspension was removed and diluted to 500 ml with either acidified, tap, or RO-purified water and used to generate 3 bottles per water type. Nine clean polysulfone water bottles were filled with the diluted antibiotic solution (3 containing each water type) with a final expected concentration of 0.80 mg sulfamethoxazole and 0.16 mg trimethoprim per milliliter. The concentration in water was extrapolated from a once-daily dose of 160 mg trimethoprim per kilogram body weight for mice, assuming a C57BL6/J mouse consumes 6 ml of water daily.[32] Bottles were inverted 4 to 5 times, stored, and sampled at the same frequency and methods described earlier.

Analysis of antibiotic stability in feed. Commercially available, closed-formula, gamma-irradiated, rodent feed (Purina 5058, Purina Mills International) was finely ground and used as the base diet to which each of the 2 antibiotic combinations was added. Feed was custom-mixed and pelleted to contain 0.15% amoxicillin and clavulanic acid powdered oral suspension (Amoxicillin and Clavulanate Potassium for Oral Suspension, Penn Labs) with a calculated final expected concentration of 800 µg amoxicillin and 150 µg clavulanic acid per gram of food. The concentration was extrapolated from the twice-daily dose of 100 mg amoxicillin per kilogram body weight for mice, assuming that a C57BL6/J mouse eats 4 g of food daily.[12,32] Commercially formulated compounded feed containing 1.2 mg sulfamethoxazole and 0.24 mg trimethoprim per gram of food was obtained (TestDiet, Purina Mills International). The concentration corresponded to 160 mg trimethoprim per kilogram body weight for mice, assuming that a C57BL6/J mouse consumes 4 g of food daily.[32] Representative process samples (approximately 10 g) of each diet were obtained after the antibiotic was mixed into the ground feed but before pelleting and immediately after pellet extrusion. Samples were express-shipped on dry ice and stored at –20 °C. The remainder of each diet (approximately 10 kg) was placed into a clear plastic bag, boxed, trucked by commercial ground carrier to a gamma irradiator, exposed to 1 to 4 mRad, and subsequently trucked to our institution. Upon arrival, approximately 30 pellets of each feed type were removed, placed into a sealed plastic bag, and stored at –20 °C. The remainder of each feed was stored in the facility's feed storage room at 21.1 ± 2.0 °C and 30% to 60% relative humidity. Thereafter, samples of 10 to 15 pellets of each diet were removed from the plastic bag monthly, with the last sample taken at 180 d after milling. Pellets were placed into airtight plastic bags and frozen at –20 °C. All feed samples were evaluated for antibiotic concentration determination by HPLC ESI–MS as described in a later section.

Determination of plasma antibiotic concentrations in mice. We used 32 male C57BL/6NCrl mice (age, 4 to 6 mo; weight, 19 to 30 g; Charles River Laboratories, Wilmington, MA) as described in a protocol approved by Memorial Sloan-Kettering Cancer Center's Institutional Animal Care and Use Committee. Mice were housed in accordance with the *Guide for the Care and Use of Laboratory Animals*[23] on a 12:12-h light:dark cycle, provided ad libitum access to acidified (pH, approximately 2.5) water, and irradiated feed (Purina Diet 5058, Purina Mills International) in solid-bottom polysulfone 'shoebox' cages containing aspen-chip bedding changed weekly and maintained in individually ventilated isolation caging (Thoren Caging Systems) unless otherwise indicated. Sentinel mice exposed to soiled bedding from the room in which the mice were housed routinely tested negative for ecto- and endoparasites, mouse hepatitis virus, Sendai

virus, Theiler mouse encephalomyelitis virus, pneumonia virus of mice, mouse parvovirus, mouse minute virus, lymphocytic choriomeningitis virus, mouse rotavirus, *Ectromelia*, reovirus type 3, K virus, mouse adenovirus, polyoma virus, cilia-associated respiratory bacillus, mouse cytomegalovirus, mouse thymic virus, Hantaan virus, *Mycoplasma pulmonis*, *Clostridium piliforme*, *Salmonella* spp., and *Citrobacter rodentium*.

Mice were housed individually for a minimum of 2 wk and were fed antibiotic-containing feed that had been milled within 30 d of use for at least 72 h prior to plasma collection. Each animal had ad libitum access to feed containing either 0.15% amoxicillin–clavulanic acid (n = 8), or 0.12% sulfamethoxazole–0.024% trimethoprim (n = 8). At 4 h after either the dark or light phase began, animals were euthanized with carbon dioxide, and cardiac blood collection was performed. We chose time points 12 h apart, occurring 4 h after lights were turned on or off to maximize the likelihood of animals exhibiting typical light:dark-cycle behaviors with minimal human interruption. Blood samples were placed into a tube containing powdered dipotassium ethylenediaminetetraacetic acid (Microtainer, Becton Dickenson and Company, Franklin Lakes, NJ) on ice and centrifuged at 2000 × *g* at 4 °C. Plasma was transferred to 0.5-ml polypropylene tubes and stored frozen at –80 °C until analyzed by HPLC ESI–MS as described in a later section.

Determination of antibiotic concentration in feed, water, and plasma samples by using HPLC ESI–MS. For each feed type, the prepelleted feed sample and samples from each collection date were granulated and mixed thoroughly (Oster blender, Jarden Consumer Solutions, Boca Raton, FL). A 1-g aliquot of the powder was transferred to a 15-ml polypropylene tube, mixed with 3 ml methanol, and agitated gently. The samples of feed containing amoxicillin and clavulanic acid were sonicated for 15 min in an ice bath, mixed, and centrifuged at 1860 × *g* for 5 min at 4 °C. The samples of feed containing trimethoprim–sulfamethoxazole were sonicated for 60 min at room temperature, mixed, and centrifuged at 1860 × *g* for 5 min at 4 °C. The supernatants from all samples were centrifuged and filtered at 13225 × *g* and 4 °C through a 45-µm nylon membrane filter (Spin-X Centrifuge Tube Filter, Corning, Corning, NY). Supernatants were diluted with methanol prior to analysis by HPLC ESI–MS.

A Gemini C18 HPLC column (5 µm, 50 × 3.0 mm; Phenomenex, Torrance, CA) was used for the separation of either amoxicillin from clavulanic acid or trimethoprim from sulfamethoxazole in water, feed, and plasma samples.[38,39,47] An inline mass spectrometer using electrospray ionization technology (Applied Biosystems, Foster City, CA) was used for the quantitative analysis of antibiotic concentrations in each sample. A set of amoxicillin–clavulanic acid and trimethoprim–sulfamethoxazole standards (Amoxicillin trihydrate, clavulanate lithium, trimethoprim, and sulfamethoxazole; USP, Rockville, MD) were used to generate standard curves with a detection limit of 5 pg/ml for clavulanic acid, 100 pg/ml for amoxicillin, 0.2 pg/ml for trimethoprim, and 0.1 pg/ml for sulfamethoxazole.

Statistical analysis. The mean antibiotic concentration in RO-purified water at time 0 was compared with the corresponding mean antibiotic concentration in tap and acidified water, and differences in mean plasma antibiotic concentrations during the night and day were analyzed by using Student *t* tests (2-sample, assuming equal variances; Excel, Microsoft Corporation, Redmond, WA). Analysis of variance (ANOVA) was performed for each antibiotic–matrix combination by using a statistical software application (JMP 6.0, SAS, Cary, NC). An overall analysis of variance was performed on data collected over 7 d within each water type for water studies or over 6 mo for food studies. If the results were significant ($P \leq 0.05$), Dunnett post hoc comparison was performed to compare the mean value at 48, 72, 120, and 168 h with that at time 0 for water studies. For feed samples, Dunnett post hoc tests were used to compare the mean value before pelleting with that at 1, 2, 3, or 4 mo after receipt.

Results

Stability of amoxicillin and clavulanic acid in water. The concentrations of both amoxicillin and clavulanic acid remaining at 0, 48, 72, 120, and 168 h in the 3 types of drinking water are illustrated in Figures 1 A to C. The expected initial concentrations of amoxicillin and clavulanic acid were 0.96 and 0.14 mg/ml, respectively. The actual concentration of amoxicillin in RO (Figure 1 A) and tap water (Figure 1 B) was relatively stable, ranging between 1.18 and 1.29 mg/ml and 1.09 and 1.22 mg/ml, respectively, over the course of the 7 d period. When added to acidified water (Figure 1 C), the concentration of amoxicillin declined immediately but subsequently remained stable at 0.43 to 0.50 mg/ml over the evaluation period. At time 0, the concentration of amoxicillin in acidified water was significantly ($P < 0.05$) lower than in RO-purified water, but there were no discernible differences in concentration in RO-purified and tap water at the same time point. At time 0, the concentrations of clavulanic acid in acidified and tap water were significantly ($P < 0.05$) lower than in RO-purified water. Clavulanic acid exhibited a statistically significant ($P < 0.05$) time-dependent decrease in concentration beginning at 72 h, with only 40% of the calculated concentration remaining at 7 d in RO-purified water (Figure 1 A). In tap water, clavulanic acid exhibited an immediate and sustained decrease in concentration, ranging from 0.02 to 0.05 mg/ml during the evaluation period (Figure 1 B). Similarly, but to a greater degree, clavulanic acid degraded in acidified water and was not detectable at any time point (Figure 1 C).

Stability of trimethoprim and sulfamethoxazole in water. Despite mixing, water bottles contained suspended particulate matter and sediment prior to sample collection. The concentrations of both trimethoprim and sulfamethoxazole acid remaining at 0, 48, 72, 120, and 168 h in the 3 types of drinking water are illustrated in Figures 2 A to C. The expected initial concentrations of trimethoprim and sulfamethoxazole were 0.16 and 0.80 mg/ml, respectively. There were no differences in mean antibiotic concentration of trimethoprim or sulfamethoxazole among the 3 water types at time 0. The concentrations of both trimethoprim and sulfamethoxazole in RO-purified, tap, and acidified water fluctuated over the 7-d period. Results ranged from 0.09 to 0.19 mg/ml trimethoprim and 0.40 to 1.43 mg/ml sulfamethoxazole in all types of water, with no clear directional trend. In acidified water, statistically significant ($P < 0.05$) decreases in sulfamethoxazole were found at 48, 72, and 120 h when compared with the concentration at time 0. In light of the high degree of variability observed, these differences likely resulted from the unequal distribution of the antibiotic suspension.

Stability of amoxicillin and clavulanic acid in feed. The concentrations of amoxicillin and clavulanic acid in feed are illustrated in Figure 3 A. The concentration of amoxicillin was approximately 60% of the expected concentration (0.80 mg/g) at all time points; however, the concentrations remained similar over the course of the 180 d testing period. Clavulanic acid concentrations measured were considerably lower (less than 10%) than expected (0.15 mg/g), and the concentration declined further after irradiation and ground shipping, decreasing to an undetectable level after 1 mo of storage.

Stability of trimethoprim and sulfamethoxazole in feed. The concentrations of sulfamethoxazole and trimethoprim in feed

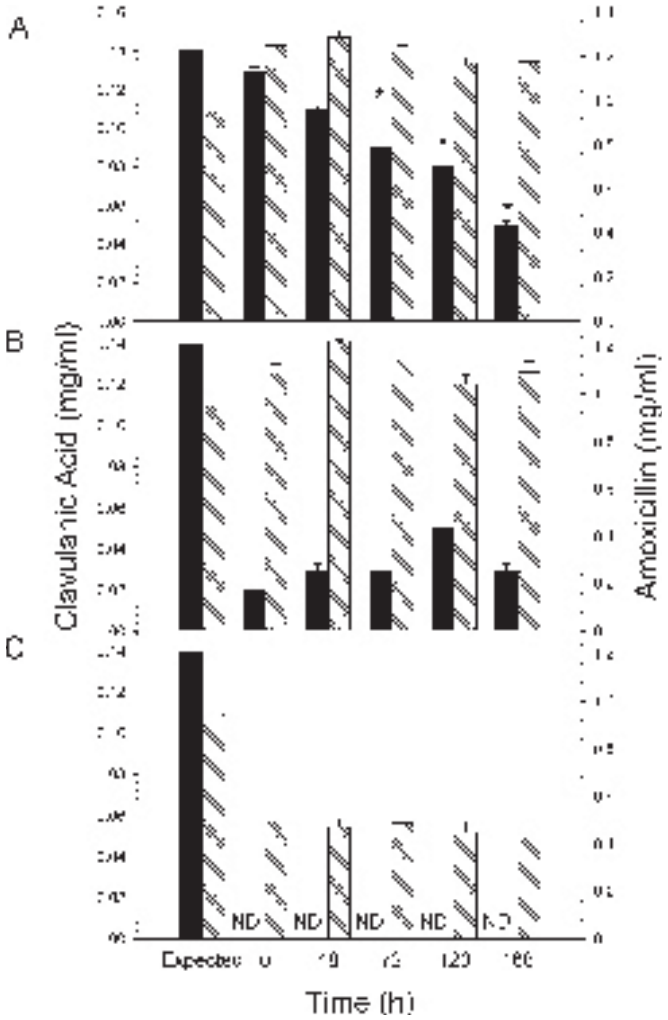

Figure 1. Concentration (mg/ml; mean ± 1 standard deviation; n = 3) of clavulanic acid (solid bar) and amoxicillin (cross-hatched bar) over 168 h in (A) reverse-osmosis-purified, (B) tap, and (C) acidified water. ND, none detected. *, $P \leq 0.05$ compared with the value at the 0-h time point.

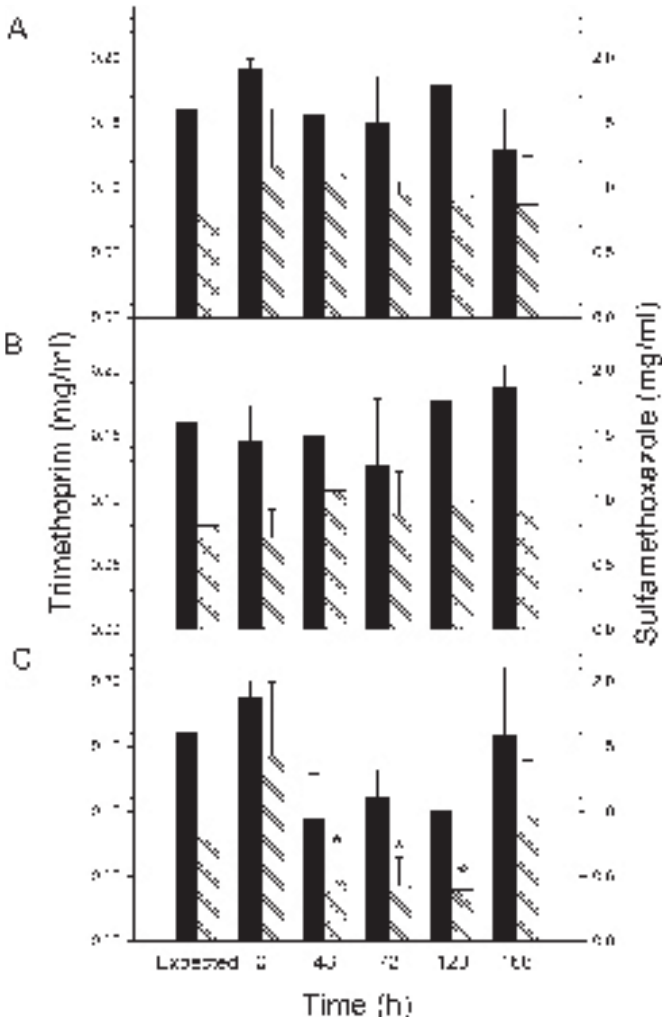

Figure 2. Concentration (mg/ml; mean ± 1 standard deviation; n = 3) of trimethoprim (solid bar) and sulfamethoxazole (cross-hatched bar) over 168 h in (A) reverse-osmosis-purified, (B) tap, and (C) acidified water. *, $P \leq 0.05$ compared with the value at the 0-h time point.

are illustrated in Figure 3 B. The measured concentrations of both sulfamethoxazole and trimethoprim were 58% and 63% less than expected (1.2 mg/g and 0.24 mg/g, respectively) after pelleting. The concentration of sulfamethoxazole varied from 0.51 to 0.74 mg/g, and the concentration of trimethoprim varied from 0.12 to 0.16 mg/g during the 6-mo period. Although not statistically significant, there was a decreasing trend in the concentrations of both antibiotics over the sampling period.

Antibiotic concentrations in plasma. The plasma concentrations of clavulanic acid, amoxicillin, trimethoprim, and sulfamethoxazole are presented in Table 1. There were no significant differences in clavulanic acid concentrations measured during the day or night. Mean plasma concentrations of amoxicillin were significantly higher at night compared with levels measured during the day. Mean plasma concentrations of trimethoprim were not significantly different when measured at night or during the day. Mean sulfamethoxazole concentrations in plasma were significantly ($P < 0.05$) increased at night.

Discussion

In this report, we examined the effects of compounding amoxicillin–clavulanic acid and trimethoprim–sulfamethoxazole into secondary matrices including tap, acidified, and RO-purified water, as well as rodent feed. Additional experiments were conducted to evaluate the plasma antibiotic concentrations in mice administered custom-compounded feed containing the respective antibiotic concentrations.

Our results demonstrated that amoxicillin and clavulanic acid rapidly degraded in acidified water. The initial concentration of amoxicillin was similar in tap and RO water, albeit slightly higher than expected. This finding was likely the result of amoxicillin's poor solubility in water. The specific mechanism(s) responsible for the concentration declines of clavulanic acid diluted in RO-purified, tap, and acidified water, and amoxicillin diluted in acidified water, are unknown. Water temperature and pH, chemical properties and reactivity, light sensitivity, and the dilution of stabilizing agents found in the suspension may each play a role in degradation. The manufacturer's storage recommendations for the reconstituted amoxicillin and clavulanic acid suspension include refrigeration and disposal after 10 d.[3] A study that evaluated the effect of storage at room temperature on a reconstituted oral suspension of amoxicillin trihydrate and clavulanic acid (Augmentin) formulated for human use (the concentration and ratio of amoxicillin–clavulanic acid are the same as used in the present study) demonstrated greater than a 10% decrease in amoxicillin concentration after 7 d and greater than a 20% decrease in the concentration of clavulanic

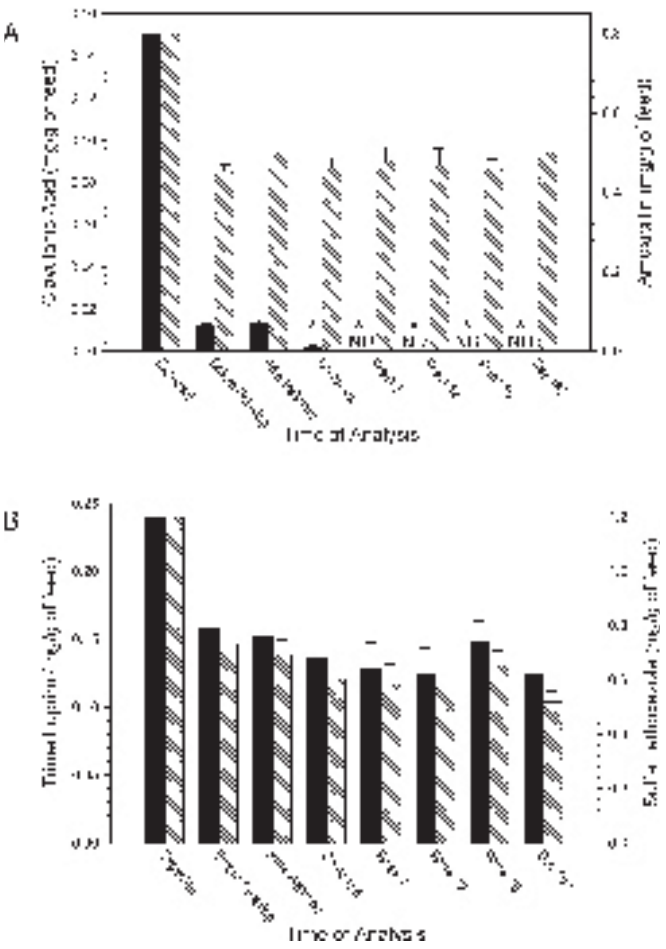

Figure 3. (A) Concentration (%; mean ± 1 standard deviation; n = 3) of clavulanic acid (solid bar) and amoxicillin (cross-hatched bar) in feed. *, $P \leq 0.05$ compared with value before pelleting. (B) Concentration (%; mean ± 1 standard deviation; n = 3) of trimethoprim (solid bar) and sulfamethoxazole (cross-hatched bar). *, $P \leq 0.05$ compared with the value before pelleting.

acid within 4 d of reconstitution.[19]

Amoxicillin is slightly soluble (4 mg/ml) in water and methanol, and its stability in water is greatest at pH 5.5; in contrast, the dissolution of amoxicillin is least between pH 4 and 6.[33,38,20] These characteristics may account for the observed stability of amoxicillin in both RO-purified (pH 6.0) and tap water (pH 6.7). Clavulanic acid is moisture-sensitive, freely soluble in water, poorly stable in aqueous solution, and is optimally stable at a pH of 6.0 to 6.3.[33,36,39] This description supports our finding that clavulanic acid was most stable in RO-purified water (pH 6.0) and is consistent with the slower decrease in concentration that occurred over time in this matrix. In contrast, a rapid decrease in clavulanic acid occurred in tap water and acidified aqueous solutions. The dilution of the inert stabilizing agents present in the manufacturer's preparation and resulting exposure of clavulanic acid to room temperature, acid pH, or solubilized or insoluble ions in tap and acidified water may result in degradation. The use of amoxicillin and clavulanic acid in rodent water bottles should be reviewed with regard to the water type used. In most circumstances, the use of amoxicillin alone would be warranted because the combination is considerably more expensive, and clavulanic acid is considerably less stable.

The concentrations of both trimethoprim and sulfamethoxazole in RO, acidified, and tap water were near expected levels but highly variable over the measurement period. We speculate that this variability resulted from our inability to adequately distribute the suspension in water. This outcome is consistent with their low solubilities in water (sulfamethoxazole is "practically insoluble"; trimethoprim solubility is 0.4 mg/ml).[20,40] The observation of particulate aggregates and sediment in some trimethoprim- and sulfamethoxazole-containing water bottles support this finding. Although the bottles were shaken prior to each sampling period, the variability in antibiotic concentration indicates that the antibiotics were not evenly distributed. The provision of these antibiotics in feed would be expected to be more uniform and allow more consistent dosing.

Because time, temperature, and gamma irradiation have previously been identified as potential causes for declines in drug concentration, we wanted to examine whether manufacturing, shipping, and storage affected the anticipated antibiotic concentrations in custom custom-compounded feed.[19,22,41] The current study demonstrated that amoxicillin, trimethoprim, and sulfamethoxazole concentrations were reduced from expected concentrations but remained stable in feed for 180 d post-milling. The modest amount of degradation noted likely resulted from the addition of water during mixing, which occurred prior to repelleting and sampling. The production of antibiotic-containing rodent feed consists of grinding a pelleted base feed, adding the desired antibiotics and water, mixing, repelleting, and drying at 90 °F.[28] Sample concentrations of clavulanic acid before and after pelleting were similar. These findings support our supposition that degradation occurred secondary to water exposure during mixing, because pre- and postpelleting samples both were collected after water was added. The specific reasons why the concentration of clavulanic acid in feed decreased further after irradiation and shipment were not identified; however, the inherent instability of clavulanic acid in the presence of water or moisture likely is involved.[46] Collectively, these results demonstrate that compounded feed may actually contain less than the anticipated concentrations of the compounded antibiotic.

Mice that received compounded antibiotics in feed exhibited both light:dark phase- and antibiotic-specific differences in plasma antibiotic concentrations. In general, individual mice exhibited variability in the respective antibiotic concentrations. This finding likely is related to the time of feed consumption relative to blood collection as well as to the cycle of the photoperiod. The plasma levels of amoxicillin and sulfamethoxazole were significantly greater in the samples collected during the dark phase. In contrast, plasma concentrations of clavulanic acid and trimethoprim were similar during the light and dark phases. Because mice are more active and ingest larger quantities of feed at night, the amoxicillin and sulfamethoxazole results were as expected.[14] However, the quantity of feed ingested is only 1 factor that can influence the plasma concentration attained. Presystemic degradation, as well as pharmacokinetic properties of these drugs, including their absorption, distribution, metabolism, and elimination characteristics, likely influenced this finding.[4,18,24] These factors may explain why minimal differences in the plasma concentration of clavulanic acid and trimethoprim were apparent.

The mean plasma concentrations measured for all 4 antibiotics were below the lower range of the minimal inhibitory concentration (MIC) for many pathogens as determined by in-vitro susceptibility testing that was conducted to support antibiotic registration for human use.[3,29] Strains of bacteria considered to be 'susceptible' to these drugs include *Escherichia coli* and *Klebsiella* spp. (MIC, 0.05 and 0.95 µg/ml for trimethoprim and

Table 1. Antibiotic concentrations in mouse plasma collected 4 h after the initiation of night and day phases of photocycle

Plasma analyte	Night (mean ± 1 standard deviation; ng/ml)	Day (mean ± 1 standard deviation; ng/ml)
Amoxicillin	104.62 ± 54.89 (n = 6)[a]	29.02 ± 18.98 (n = 8)[b]
Clavulanic acid	4.43 ± 2.08 (n = 6)[a]	6.66 ± 3.97 (n = 8)
Trimethoprim	0.11 ± 0.05 (n = 8)	0.10 ± 0.02 (n = 8)
Sulfamethoxazole	3.09 ± 1.39 (n = 8)	0.27 ± 0.34 (n = 8)[c]

[a]Two samples lost due to technical problems.
[b]$P < 0.003$ versus value for night sample.
[c]$P < 0.001$ versus value for night sample.

sulfamethoxazole, respectively), gram-negative enteric aerobes (MIC, ≤8 and 4 µg/ml for amoxicillin and clavulanic acid, respectively), nonmeningitidis *Streptococcus pneumoniae* and *Staphylococcus* spp. (MIC, ≤2 and 1 µg/ml and ≤4 and 2 µg/ml, respectively, for amoxicillin and clavulanic acid). The plasma antibiotic concentrations measured fell well below these MIC ranges; however, plasma levels attained may inhibit bacteria at the low levels attained, given that dilutions were not performed to determine the absolute MICs.[3,29]

These results suggest that mice routinely administered antibiotics via feed may be receiving a suboptimal or nonefficacious antibiotic dose and may be at risk for emergence of antibiotic resistance.[7,8,11,16,19,45,46] The MICs of individual murine pathogen(s) of concern should be taken into account to definitively ensure that efficacious plasma levels and treatment times are achieved and maintained.[44] The American Veterinary Medical Association's position statement on compounding and the Food and Drug Administration allow, but limit, compounding to drugs for which both safety and efficacy have been demonstrated in the target species.[4,34,35] In addition, dosing regimens should be selected to minimize the potential of adverse effects. A recent report demonstrated that mice given trimethoprim and sulfamethoxazole exhibit decreased breeding efficiency and hypothyroidism.[1]

Appreciable levels of clavulanic acid were not expected in the plasma, because we noted that its concentration in feed decreased markedly after shipment to the extent that the drug was undetectable at 30 d after milling. The low plasma concentrations present may reflect the small amount of residual drug, because feed used for plasma antibiotic concentration was manufactured within a month of its administration. In addition, the concentration measured in feed may have been affected by extraction efficiency. However, preliminary extraction efficacy studies with spiked feed did not suggest a marked matrix effect (data not shown). We further expected that the concentrations of trimethoprim and clavulanic acid in plasma to be greater in samples collected during the dark phase of the light cycle. Pharmacokinetic properties of these drugs, including their absorption, distribution, metabolism, and elimination characteristics, likely influenced this finding.

Effective administration of antibiotics is required for the eradication of pathogenic bacteria and is an important factor in the emergence of antibiotic-resistant strains. Drug-resistant organisms proliferate in hospitals because of the dense population of immunosuppressed patients, personnel acting as fomites, and the widespread use of antibiotics.[26] Similar conditions are found in laboratory animal facilities that house high-density immunosuppressed rodents and that employ husbandry and research staff who may serve as fomites transmitting infectious agents. Although widespread drug-resistant laboratory animal pathogens have not yet been identified, the selective pressure of widespread and suboptimal antibiotic use increases the likelihood of developing a resistant organism.[7,8,11,16,27,45]

Guidelines for medicated food or water formulations that minimize suboptimal plasma antibiotic concentrations may more appropriately be based on consumption during the period when mice are least likely to ingest food or water voluntarily. Given our findings, the concentration of a specific antibiotic should be adjusted in the matrix to attain the plasma antibiotic concentration needed for optimal bactericidal activity over a 24-h period.

In summary, our results indicate that the concentrations of antibiotics can vary markedly when custom-compounded in feed and water. Mice given antibiotics in food may not achieve plasma concentrations that are likely necessary for optimal efficacy. Custom-compounded water or feed for laboratory rodents should be examined closely prior to use to ensure that the compounded medication retains its potency, stability, and efficacy. In addition, susceptibility testing of murine pathogens should be conducted to ensure that the plasma antibiotic concentration attained and its concentration over time are effective to ensure current and future laboratory rodent health.

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References

1. **Altholtz LY, LaPerle KMD, Quimby FW.** 2006. Dose-dependant hypothyroidism in mice induced by commercial trimethoprim–sulfamethoxazole rodent feed. Comp Med **56:**395–401
2. **American Veterinary Medicine Association** [Internet]**.** 2005. AVMA position statements: compounding [cited 26 Oct 2006]. Available from http://www.avma.org/issues/drugs/compounding/compounding.asp.
3. **Amoxicillin–Clavulanate Potassium Powder for Oral Suspension and Chewable Tablets** [Package insert]. 2005. Philadelphia PA: Penn Labs.
4. **Chesajimenez J, Peris JE, Torresmolina F, Granero L.** 1994. Low bioavailability of amoxicillin in rats as a consequence of presystemic degradation in the intestine. Antimicrob Agents Chemother **38:**842–847.
5. **Cohn LA, Weber AF, Kaplan M, Smith AL.** 2000. Effect of chloramphenicol administration in drinking water on predictable steady-state serum concentrations of the drug in mice. Comp Med **50:**56–58.
6. **Conole J, Wilkinson MJ, McKellar QA.** 2003. Some observations on the pharmacological properties of ivermectin during treatment of a mite infestation in mice. Contemp Top Lab Anim Sci **42(4):**42–44.
7. **Drusano GL.** 2003. Prevention of resistance: a goal for dose selection for antimicrobial agents. Clin Infect Dis **36(Suppl 1):** S42–S50.
8. **Drusano GL, Louie A, Deziel M, Gumbo T.** 2006. The crisis of resistance: identifying drug exposures to suppress amplification of resistant mutant subpopulations. Clin Infect Dis **42:**525–532.

9. **Foltz CJ, Fox JG, Yan L, Shames B.** 1996. Evaluation of various oral antimicrobial formulations for eradication of *Helicobacter hepaticus*. Lab Anim Sci **46:**193–197.
10. **Goelz MF, Thigpen JE, Mahler J, Rogers WP, Locklear J, Weigler BJ, Forsythe DB.** 1996. Efficacy of various therapeutic regimens in eliminating *Pasteurella pneumotropica* from the mouse. Lab Anim Sci **46:**280–285.
11. **Gould IM, Mackenzie FM.** 2002**.** Antibiotic exposure as a risk factor for emergence of resistance: the influence of concentration. Symp Ser Soc Appl Microbiol **92:**78S–84S
12. **Hawk CT, Leary SL, Morris TH.** 2005. Anti-infectives. In: Hawk CT, Leary SL, Morris TH, editors. Formulary for laboratory animals, 3rd ed. Ames (IA): Blackwell Publishing. p 71–106.
13. **Hayes JM, Eichman J, Katz T, Gilewicz R.** 2003. Stability of florfenicol in drinking water. J AOAC Int **86:**22–29.
14. **Jacoby RO, Fox JG, Davisson M.** 2002. Biology and diseases of mice. In: Fox JG, Anderson LC, Loew FM, Quimby FW, editors. Laboratory animal medicine, 2nd ed. San Diego (CA): Academic Press. p 35–120.
15. **Jury J, Gee LC, Delaney KH, Perdue MH, Bonner RA.** 2005. Eradication of *Helicobacter* spp. from a rat breeding colony. Contemp Top Lab Anim Sci **44(4):**8–11.
16. **Lewis R** [Internet]**.** 1995. The rise of antibiotic-resistant infections. FDA consumer magazine [cited 10 Oct 2006]. Available from http://www.fda.gov/fdac/795_toc.html.
17. **Lust E.** 2004. Compounding for animal patients: contemporary issues. J Am Pharm Assoc (2003) **44:**375–384.
18. **McKellar QA, Sanchez Bruni SF, Jones DG.** 2004. Pharmacokinetic–pharmacodynamic relationships of antimicrobial drugs used in veterinary medicine. J Vet Pharmacol Ther **27:**503–514.
19. **Mehta AC, Hart-Davies S, Payne J, Lacey RW.** 1994. Stability of amoxycillin and potassium clavulanate in co-amoxiclav oral suspension. J Clin Pharm Ther **19:**313–315.
20. **Merck Index** [Internet]. c2007. Merck monograph no. 09709: trimethoprim [cited 5 May 2007]. Available from http://themerckindex.cambridgesoft.com/themerckindex.
21. **Mickley AG, Hoxha ZH, Biada JM, Kenmuir CK, Bacik SE.** 2006. Acetaminophen self-administered in the drinking water increases the pain threshold of rats (*Rattus norvegicus*). JAALAS **45(5):**48–54.
22. **Moore TD, Horton R, Utrup LJ, Miller LA, Poupard JA.** 1996. Stability of amoxicillin–clavulanate in BACTEC medium determined by high-performance liquid chromatography and bioassay. J Clin Microbiol **34:**1321–1322.
23. **National Research Council.** 1996. Guide for the care and use of laboratory animals. Washington (DC): National Academy Press.
24. **Papich MG.** 2005. Drug compounding for veterinary patients. AAPS J **7:**E281–E287.
25. **Plumb DC.** 1994. Do veterinarians have the training and equipment to compound drugs? J Am Vet Med Assoc **205:**268–269.
26. **Rybak MJ.** 2006. Pharmacodynamics: relation to antimicrobial resistance. Am J Med **119(6 Suppl 1):**S37–S44; discussion S62-S70.
27. **Smith SV, Gould IM.** 2004. Optimization of antibiotic dosing schedules in light of increasing antibiotic resistance. Expert Rev Anti Infect Ther **2:**227–234.
28. **Stroebel V.** 2006. Personal communication.
29. **Sulfamethoxazole and Trimethoprim Oral Suspension [**Package insert**].** 2005. Amityville (NY): Hi-Tech Pharmacal.
30. **Sumano H, Hernandez L, Gutierrez L, Bernda-Bernad MJ.** 2005. Sustained availability of trimethoprim in drinking water to achieve higher plasma sulphonamide–trimethoprim antibacterial activity in broilers. Br Poult Sci **46:**114–118.
31. **Taconic Labs** [Internet]**.** 2007. Notification to all recipients of p47 PHOX knockout Mice NIAD emerging Line #004227-M [cited 3 March 2007]. Available from http://www.taconic.com/wmspage.cfm?parm1=1342.
32. **Tordoff MG, Bachmanov AA** [Internet]**.** 2001. Food intakes, water intakes, and spout side preferences. MPD:63 Mouse Phenome Database [cited 14 June 2005]. Available from http://www.jax.org/phenome.
33. **Tsuji A, Nakashima E, Hamano S, Yamana T.** 1978. Physiochemical properties of amphoteric β-lactam antibiotics: stability, solubility, and dissolution behavior of amino penicillins as a function of pH. J Pharm Sci **67:**1059–1066.
34. **United States Code of Federal Regulations** [Internet]**.** 2006. Title 21 part 530 §30 Extralabel drug use in animals [cited 1 Nov 2006]. Available from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=530.
35. **United States Food and Drug Administration** [Internet]**.** 2003. Compliance policy guide. Compounding of drugs for use in animals [cited 30 Oct 2006]. Available from http://www.fda.gov/ora/compliance_ref/cpg/cpgvet/cpg608-400.html.
36. **United States Pharmacopeiea** [Internet]. c2007. US Pharmacopeiea description and solubility [cited 25 Oct 2006]. Available from http://www.uspnf.com/uspnf/pub/index?usp=29&nf=24&s=2.
37. **United States Pharmacopeiea** [Internet]. c2007. 1075 Good compounding practices [cited 31 Oct 2006]. Available from http://www.uspnf.com/uspnf/pub/data/v29242/usp29nf24s2_c1075.xml.
38. **United States Pharmacopeiea** [Internet]. c2007. USP monographs: amoxicillin [cited 13 Oct 2006]. Available from http://www.uspnf.com/uspnf/pub/data/v29242/usp29nf24s2_m4100.xml.
39. **United States Pharmacopeiea** [Internet]. c2007. USP monographs: clavulanate potassium [cited 13 Oct 2006]. Available from http://www.uspnf.com/uspnf/pub/data/v29242/usp29nf24s2_m1800.xml.
40. **United States Pharmacopeiea** [Internet]. c2007. USP material safety data sheet: sulfamethoxazole [cited 2007 May 5]. Available from http://www.usp.org/pdf/EN/referenceStandards/msds/1631001.pdf.
41. **Valvo L, Manna L, Alimenti R, Alimonti S, Bertocchi P, Ciranni E.** 1999. Amoxicillin sodium–potassium clavulanate: evaluation of gamma radiation induced effects by liquid chromatography on both the individual drugs and their combination. J Pharm Biomed Anal **21:**9–14.
42. **Walzer PD, Brunck J, Steele P, White M, Linke MJ, Sidman CL.** 1997. Immunodeficient and immunosuppressed mice as models to test anti-*Pneumocystis carinii* drugs. Antimicrob Agents Chemother **41:**251–258.
43. **Walzer PD, Kim CK, Linke MJ, Pogue CL, Huerkamp MJ, Chrisp CE, Lerro AV, Wixson SK, Hall E, Shultz LD.** 1989. Outbreaks of *Pneumocystis carinii* pneumonia in colonies of immunodeficient mice. Infect Immun **57:**62–70
44. **Weber DJ, Tolkoff-Rubin NE, Rubin RH.** 1984. Amoxicillin and potassium clavulanate: an antibiotic combination. Mechanism of action, pharmacokinetics, antimicrobial spectrum, clinical efficacy, and adverse effects. Pharmacotherapy **4:**122–136.
45. **Weber JT.** 2005. Appropriate use of antimicrobial drugs: a better prescription is needed. J Am Med Assoc **294:**2354–2356.
46. **Woodnutt G.** 2000. Pharmacodynamics to combat resistance. J Antimicrob Chemother **46(Suppl T1):**25–31.
47. **Woodnutt G, Berry V.** 1999. Two pharmacodynamic models for assessing the efficacy of amoxicillin-clavulanate against experimental respiratory tract infections caused by strains of *Streptococcus pneumoniae*. Antimicrob Agents Chemother **43:**29–34.
48. **Yoon KH, Lee SY, Kim W, Park JS, Kim HJ.** 2004. Simultaneous determination of amoxicillin and clavulanic acid in human plasma by HPLC-ESI mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci **813:**121–127.