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Stability of Free Available Chlorine Levels in Dilute Sodium Hypochlorite Solutions over a 6-Week Period

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Animal care and use programs commonly use chlorine and chlorine-based disinfectants to help prevent facility acquired infections in animals. The Department of Comparative Medicine (DCM) at Oregon Health and Science University (OHSU) follows the Centers for Disease Control and Prevention (CDC) disinfection guidelines for preparing and storing these disinfectants. DCM prepares bottles of dilute solutions of sodium hypochlorite (that is, commercial bleach) daily. In this study, we tested whether dilute bleach solutions, as prepared following the DCM protocol, remained stable under real-world practice conditions for up to 6 wk. We tested 4 groups of spray bottles filled with 0.5% bleach solutions in these experiments. Specifically, we sprayed 2 groups of bottles daily to mimic use while 2 other groups of bottles were not sprayed. We then measured free available chlorine (FAC) using 2 methods, spectrophotometry and colorimetric strips. All 4 test groups showed stable maintenance of FAC concentration for the length of the experiment. Mean FAC loss from baseline levels was not significantly different in the group of bottles not sprayed daily (6% for group 2 at week 5 compared with 7% for Group 4 at week 6). All bottles in Groups 1 and 3 measured by colorimetric strips showed concentrations at or near 5000 mg/L at all weekly time points throughout the experiment. This study shows that 0.5% sodium hypochlorite solutions stored and used in a standard rodent housing room and sprayed daily will maintain acceptable FAC concentrations for at least 5 to 6 wk, perhaps longer. In addition, we report that colorimetric strips may be a useful and accessible quality control tool for testing freshly prepared solutions at regular intervals. We conclude that sodium hypochlorite solutions can be prepared on a weekly, biweekly, or monthly basis with no loss in disinfection effectiveness.

Abbreviations: DCM, Department of Comparative Medicine; OHSU, Oregon Health and Science University; CDC, Centers for Disease Control and Prevention; FAC, free available chlorine

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Introduction

The prevention of animal infection through the accidental introduction or propagation of pathogens is a high priority in a research setting. Infections in research animals can spread quickly and confound studies, and may require diagnostic tests, isolation, or treatment of infected animals. Some pathogens may require colony depopulation to ensure the long-term health of animals in a facility. Laboratory animal medicine also presents unique challenges to infection control as animals with varying degrees of immune status are routinely used in research experiments. Immunocompromised animals are more susceptible to infection and are more likely to require euthanasia due to poor health.

Infectious microorganisms can be introduced easily through mishandling or improper disinfection of animal housing or care items, but can be successfully avoided using disinfection protocols from the CDC with Environmental Protection Agency (EPA)-registered disinfectants, such as alcohols, chlorine and chlorine compounds, formaldehyde, glutaraldehyde, hydrogen peroxide, iodophors, phenolics and quaternary ammonium compounds.[18] Disinfectant effectiveness (that is, spectrum of activity, speed of action) must be balanced against health risks and financial costs. Some disinfectants, while effective, may cause animal toxicity, including lung and brain injury from fume inhalation or occupational health hazards such as fume inhalation, dermal or ocular irritation or burns, the cost of disinfectant and of the labor required to prepare and use it must also be considered.[18] Moreover, the CDC offers guidelines for disinfectant use in human healthcare facilities, but these may not be appropriate for animal research settings. CDC guidelines on disinfectant concentrations and use may be excessively stringent for carefully controlled laboratory environments, causing unnecessary expense to programs and potential harm to animals and staff. Alternatively, disinfectant storage conditions in research environments may be harsher than healthcare environments, speeding degradation and decreasing effectiveness more quickly and suggesting that more stringent protocols are needed.

Many care and use programs for research animals follow CDC guidelines and use chlorine-based disinfectants, particularly sodium hypochlorite (bleach), for routine workstation and animal equipment disinfection. In this study, we evaluated the standard operating procedure (SOP) for sodium hypochlorite disinfection in the DCM at OHSU. Per departmental SOP, we prepared a 0.5% (5000 mg/L) working solution daily for the disinfection of rodent workstations, transfer forceps, equipment and surfaces that may contact rodents, their caging or their waste. This SOP is based on current CDC guidelines and represents current use of the guidelines in a representative animal care and use program.

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The DCM uses bleach because of its reasonably broad spectrum of antimicrobial activity, low toxicity and levels of toxic residues, lack of sensitivity to water hardness, quick action, and low cost. However, some small but notable differences between CDC recommendations and current practice argue for a more stringent protocol. For example, the CDC Disinfection Guidelines state that, "hypochlorite solutions in tap water at a pH >8 stored at room temperature (23°C) in closed, opaque, plastic containers can lose up to 40-50% of their free available chlorine (FAC) over one month."[18] OHSU DCM staff store dilute sodium hypochlorite solutions in opaque spray bottles in animal care rooms or at animal care stations in spaces with fluctuating temperature and light exposure. In addition, tap water pH is not routinely measured when DCM staff prepare dilute solutions. However, current OHSU DCM protocols could be overly stringent, particularly with regard to the requirement to prepare solutions fresh daily, which increases risk to DCM staff and costs for reagent and labor. The potential harms of sodium hypochlorite use are highest for the technical staff who prepare working dilutions from purchased commercial bleach (5000 mg/L); these risks include potential for ocular irritation and chemical burns, metal corrosion at high concentrations (>500 ug/L), potential release of chlorine gas when mixed with ammonia or acid, and low relative stability over very long time periods.[18] Therefore, we sought to determine whether the current CDC guidelines are appropriate for use in a laboratory animal setting.

In this study, we tested whether dilute bleach solutions, as prepared using the DCM protocol, remained stable under real-world practice conditions for up to 6 wk. The overall goal of this project was to provide practical guidance in use and storage of bleach solutions in research animal settings and to provide programs with technical information on testing options available for determining the stability of sodium hypochlorite used under specific storage and use conditions.

Prior studies have shown that the decomposition rate of sodium hypochlorite is mainly dependent on pH, concentration, temperature, and ambient light exposure.[5,8-11,15-17] The ideal storage conditions to maximize sodium hypochlorite solution stability would include maintaining a solution at a pH of 9 to 11, at temperatures below 30 °C, and in an opaque bottle with little to no ambient light exposure.[4-6] One predictive model of FAC loss showed that a 1.25% commercially available sodium hypochlorite solution, stabilized to a pH of 11.9, degrades 10% after 660 d at 25 °C.[13] However, this model does not consider real-world variables. We added to the existing literature by testing the role of air introduction into bleach spray bottles due to daily use (via using spray bottles to mimic daily practice) and by testing for the loss of FAC over a 6-wk period when stored and used in an active laboratory animal care facility. We used 2 methods to determine FAC concentrations—spectrophotometry, a highly quantitative approach that may not be available to some programs, and colorimetric strips, which are widely available and easy to use but semiquantitative. We hypothesized that we would find be no significant difference in FAC concentrations over a 6-wk period, and that a difference in FAC concentrations would not develop between bottles sprayed daily and those that are not. If true, these results would indicate that dilute bleach solution remains stable for up to 6 wk under active use conditions, suggesting that less frequent solution preparation would be acceptable, representing a saving of both cost and effort.

Materials and Methods

Experimental design. We tested 4 groups of 3 spray bottles each (total of 12 bottles) of sodium hypochlorite solution (Figure 1). Two of the 4 groups were sprayed daily to mimic daily use; the remaining 2 groups were not sprayed (Figure 1). We then used 2 testing methods to evaluate FAC available chlorine concentrations at weekly time points: spectrophotometry and colorimetric chlorine strips.

The plastic spray bottles we used in these experiments were 32-ounce, white, opaque, high-density polyethylene bottles (Wesco Supply, Long Beach, CA) with 9 to 3/4" Adjust-O-Spray triggers (Wesco Supply, Long Beach, CA). To closely mimic practice conditions in rodent housing rooms, we stored bottles in a vacant rodent housing room and maintained these rooms on a 12:12-h light: dark cycle, at 19.4 to 22.8 °C and 30% to 70% relative humidity (as recorded by a thermo-humidity meter) for the duration of the study. Because light exposure has been previously shown to contribute to sodium hypochlorite degradation, we ensured maximum light exposure to simulate degradation under the most extreme practice conditions.[2,5,8,9,11,16,17] To accomplish this, we positioned bottles in a grid in the middle of a stainless-steel table, approximately 1 m high, directly below a lighting banister fitted with a compact fluorescent bulb (Figure 2). We adjusted the exact positioning under the light banister to expose all bottles to the same average light intensity (lux), as confirmed by measuring the center of the bottle grouping with a LX1330B lux meter (Sinometer, ShenZen, China).

Sodium hypochlorite solutions. In initial experiments, each bottle was filled with a 0.5% (5000 mg/L) sodium hypochlorite solution using the current DCM protocol. The solutions were made by diluting 83 mL of 6% Pure Bright Germicidal Ultra Bleach (KIK International LLC, Concord, Ontario, Canada) with 917 mL of tap water (1:12 dilution) using 250 mL and 1000 mL polypropylene graduated cylinders. Initial spectrophotometry results indicated that this protocol did not reliably produce a 5000 mg/L solution (data not shown). Therefore, in subsequent experiments, we adjusted the dilution to achieve a starting concentration of 5000 mg/L, as confirmed by spectrophotometry. We empirically determined that the starting concentration of the purchased bleach product was 4.5%, rather than the expected 6%. Thus, the final protocol used 111 mL of bleach product and 889 mL of tap water (1:9 dilution) to achieve a final concentration of 5000 mg/L.

Mimicking daily use of bottles. We sprayed bottles in Groups 1 and 2 (Figure 1) to mimic the effects of volume depletion and introduction of room air into spray bottles as a result of daily

Group	Bottle #	FAC Measuring Method	Storage Conditions
1	1	Colorimetric Chlorine Strips	Sprayed Daily
	2		
	3		
2	4	Spectrophotometry	
	5		
	6		
3	7	Colorimetric Chlorine Strips	Not Sprayed Daily
	8		
	9		
4	10	Spectrophotometry	
	11		
	12		

FAC, Free Available Chlorine

Figure 1. Experimental design by group, bottle identification, testing method, and storage conditions. The study design included 2 groupings of 6 bottles each: the first group (group 1 and 2) was sprayed daily to mimic use and the second group (group 3 and 4) was not sprayed, to test the role of air introduction in sodium hypochlorite degradation. Three bottles in each group (group 2 and 4) were monitored for free available chlorine (FAC) using spectrophotometry and three by colorimetric strips (group 1 and 3).

Figure 2. Set-up of the table and bottles within the empty rodent housing room. Twelve bottles (3 rows by 4 columns) were positioned at the center of a stainless-steel table, approximately 1 m tall, directly below the lighting banister. The exact positioning was adjusted until all bottles were exposed to the same average light intensity (lux), as confirmed by a light meter.

use. On days 0 to 42 of the experiment, we gently swirled each Group 1 and Group 2 bottle to thoroughly mix the solution and adjusted the nozzle until it produced a fine mist before continuing to spray for a total of 50 times per weekday based on our estimation of a standard workweek and the number of sprays required to saturate the working surface of an animal transfer station.

Sample collection for pH and FAC analysis. We sampled solutions weekly for the 6 wk duration of the experiment. We gently swirled bottles before twisting off the trigger spray. We transferred a 10 mL sample by a plastic serological pipette into a labeled 25 mL glass sample cell (Hach, Loveland, CO). We replaced the trigger spray and rinsed the serological pipette with tap water before proceeding to the next bottle. We collected sample cells into an enclosed cardboard box at room temperature until all samples were ready for analysis. We then measured the pH for all samples using an Orion 720A Plus digital pH meter (Fisher Scientific, Waltham, MA). The pH meter has a default resolution of 3 significant digits and an accuracy of ±0.002 pH. We calibrated the unit at the beginning of each time point using 3 standard solutions (pH 4, pH 7, pH 10). We adjusted the samples to a pH between 6 and 7 using 1N sulfuric acid before proceeding to FAC analysis.

Several methods can be used to measure and monitor FAC. The selection of specific methods depends on ease, precision and accuracy, available resources, and equipment. Semiquantitative methods include colorimetric strips that convert changes in FAC concentration to a visual color comparator. These strips are useful for nonregulatory reporting and for spot checking. Unfortunately, reading the color changes is subjective and is influenced by the light source and individual ability to judge subtle differences in color.[14] More accurate methods include spectrophotometry and iodometric titration. Spectrophotometry uses a photometer to accurately measure colorimetric changes that correspond with FAC concentrations. However, spectrophotometry is technically more challenging and requires equipment that ranges in price from a few hundred dollars to several thousand dollars.[14]

We measured the FAC concentrations in samples from Groups 2 and 4 using a DR2000 spectrophotometer (Hach, Loveland, CO) set to method 80 in accordance with USEPA DPD Method 8021.[3] This spectrophotometer has a resolution of 2 significant digits. Because we expected concentrations of FAC chlorine to exceed the upper range of the instrument (2.00 mg/L), the samples were diluted by transferring 4 µL of sample to a 10 mL glass sample cell (Hach, Loveland, CO) filled with deionized water. The diluted sample was then vortexed before proceeding with the remaining instructions for method 8021. A 1:2500 dilution factor was used to provide the highest resolution between expected readout values while minimizing error introduced by dilution. All samples were analyzed in triplicate.

FAC concentrations were estimated in samples from Groups 1 and 3 by using Bartovation extra high-level chlorine test strips (Queens, NY). These test strips detect FAC at the following concentrations: 0, 1000, 2500, 5000, 7500, 10000. One unblinded individual tested the samples following the manufacturer's protocol. An individual test strip was briefly submerged in the solution sample for one second, removed, and left on the benchtop for 30 s. The test strip color was evaluated within the next 10 s. All samples were analyzed in triplicate.

Statistical analysis. Statistical analysis was performed using Prism 9.1.2 (GraphPad Software, San Diego, CA). Group data were the mean ± 1 SD and individual data as the mean ± 1 SEM. Multiple unpaired *t* tests were used to compare group data for pH and FAC. Statistical significance was defined as a *P* value of less than 0.05 for all analyses.

Results

Environmental parameters. Environmental parameters measured in the experimental space (laboratory animal housing room) included room temperature, relative humidity, and light intensity (Figure 3A-C). Room temperature ranged from 18.9 to 21.1 °C, with a mean temperature of 21 °C (SD = 0.8) (Figure 3A). Relative humidity (as measured with a monitor placed at bottle height) ranged from 17% to 32%, with a mean of 20% (SD = 4) (Figure 3B). Light intensity (as measured with a lux meter centered over experimental bottles) ranged from 345 to 359 lx, with a mean of 352 lx (SD = 3) (Figure 3C). All environmental parameter measurements were within ranges commonly accepted as normal in animal housing units, with the exception of relative humidity, which was lower than usual in our experiment. However, relative humidity is commonly measured in animal spaces at the level of building ducts, rather than at the table height level measured here.

pH. pH values were generally stable in all bottles over the course of the experiment, despite slight decreases with time (Figure 4). Bottles in Groups 1 and 2 (Sprayed Daily) showed a mean baseline pH of 11.5 (SD = 0.05), which fell to 11.0 (SD = 0.06) by week 5 (Figure 4). pH values were not recorded for bottles in Groups 1 and 2 beyond week 5 because daily spraying completely depleted the bottles' contents. Bottles in Groups 3 and 4 (not sprayed daily) showed a mean baseline pH of 11.5

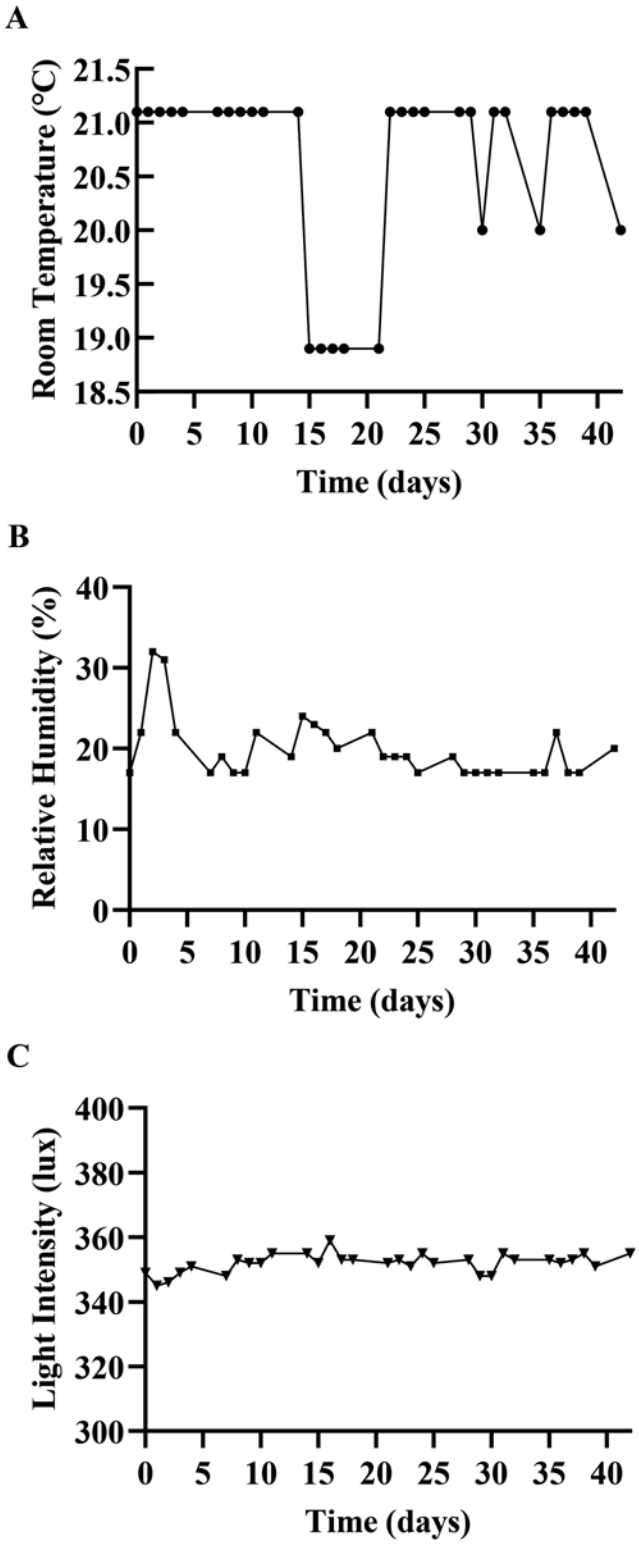

Figure 3. Environmental parameters recorded as (A) room temperature (°C), (B) relative humidity (%), and (C) light intensity (lux). The room temperature and relative humidity were measured using a thermo-humidity meter placed at the level of the bottles. (A) Room temperature remained relatively stable throughout the experiment except for a 7-d period where the temperature dropped below the lower limit (19.4 °C) to 18.9 °C. (B) Relative humidity varied between 17% and 32% throughout the experiment. (C) Light intensity was measured daily with a light meter and was very stable throughout the experiment with an average of 352 lx.

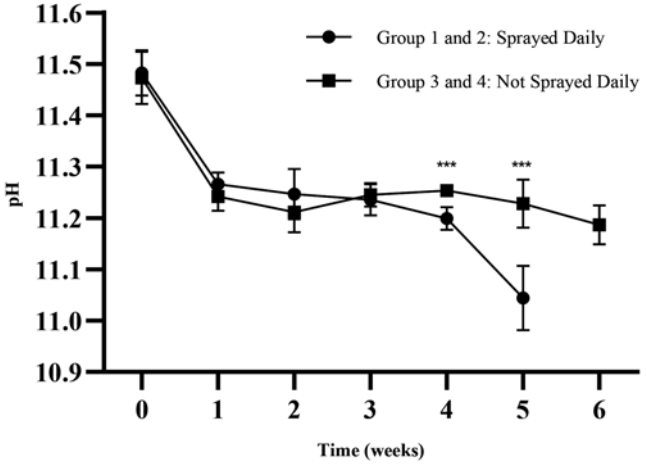

Figure 4. pH of solutions that were sprayed (Group 1 and 2) or not sprayed daily (Group 3 and 4) over time (mean ± 1 SD [error bars]). The pH values were generally stable throughout the experiment despite a slight decrease with time. Groups 1 and 2 (sprayed daily) had a baseline pH of 11.5 that decreased to 11.0 by week 5. Groups 3 and 4 (not sprayed daily) had a baseline pH of 11.5 that decreased to 11.2 by week 6. There was a statistically significant difference ($P < 0.05$) at weeks 4 and 5. Group 1 and 2 bottles (sprayed daily) had pH values of 11.2 and 11.0 compared with Group 3 and 4 bottles (not sprayed daily) that had pH values of 11.3 and 11.2 on weeks 4 and 5, respectively. Note that daily spraying completely depleted the bottle contents of Group 1 and 2 by week 5.

(SD = 0.05) that fell to 11.2 (SD = 0.04) by week 6 (Figure 4). Unpaired *t* tests comparing sprayed daily (combined data from Groups 1 and 2) and not sprayed daily (combined data from Groups 3 and 4) conditions showed a statistically significant difference ($P < 0.05$) at weeks 4 and 5, although effect sizes were small and unlikely to reflect functionally significant differences in disinfectant chemistry. Specifically, mean pH values for sprayed daily bottles were 11.2 and 11.0 for weeks 4 and 5, respectively. Mean pH values for unsprayed daily bottles were 11.3 and 11.2 for weeks 4 and 5, respectively.

Free available chlorine. Spectrophotometry measurements of FAC for 2 groups of bottles (Group 2 [sprayed daily] and Group 4 [not sprayed daily]) revealed that initial FAC levels were near the target starting concentration of 5000 mg/L; measured FAC concentrations were 4880 mg/L (SD = 23) in Group 2 and 4950 mg/L (SD = 12) in Group 4 (Figure 5C). FAC concentration fell slightly with time for all bottles, reaching a final concentration of 4600 mg/L (SD = 21) for Group 2 (at week 5) and 4630 mg/L (SD = 31) for Group 4 (at week 6) (Figure 5A-B). As noted above, Sprayed Daily bottles were empty by the end of week 5, due to daily spraying. Mean FAC loss from baseline levels was not significantly different between the 2 groups (6% for Group 2 at week 5 compared with 7% for Group 4 at week 6).

Colorimetric chlorine strips were used to evaluate FAC levels for the remaining 2 groups of bottles: Group 1 (sprayed daily) and Group 3 (not sprayed daily). All bottles in both groups showed concentrations at or near 5000 mg/L at all weekly time points throughout the experiment (5 wk for Group 1 and 6 wk for Group 2).

Discussion

This study evaluated an existing CDC guideline-based SOP for sodium hypochlorite solution preparation for laboratory animal use. The data showed that FAC levels remained stable for up to 6 wk. In addition, spray bottles in active use were de-

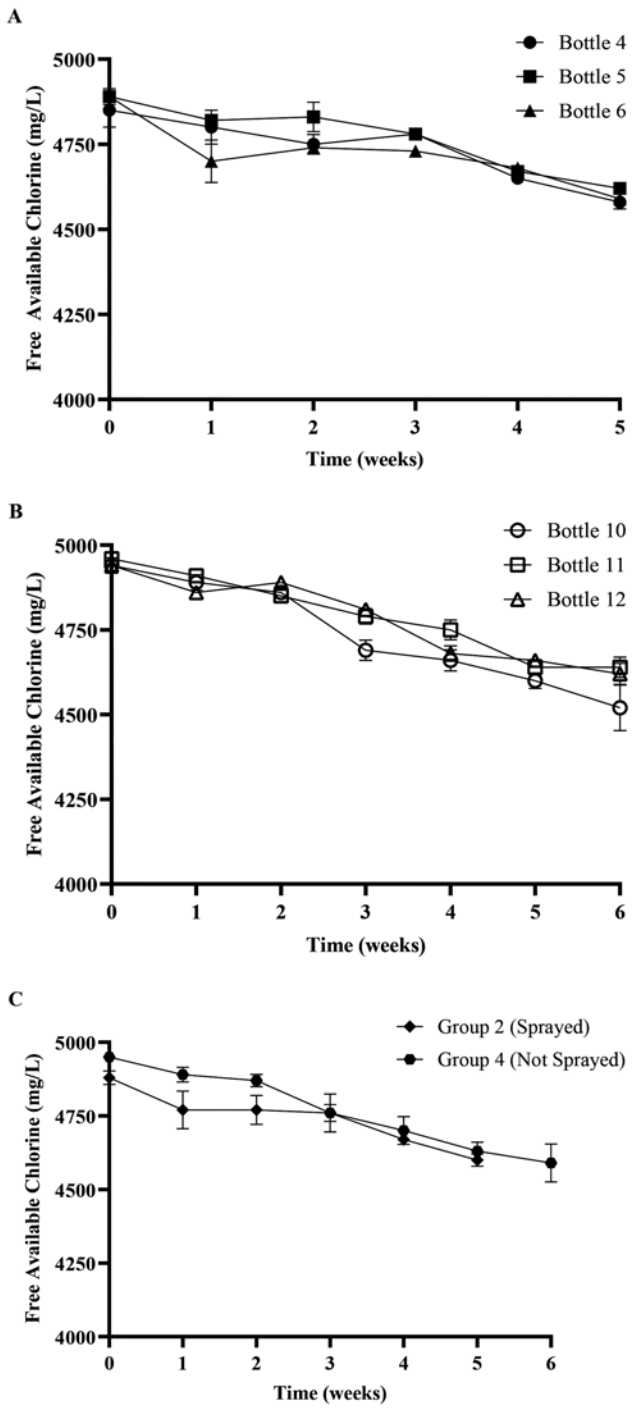

Figure 5. Degradation curves of dilute sodium hypochlorite solutions that were (A) Sprayed daily or (B) Not Sprayed Daily (mean ± 1 SEM [error bars]). (C) Group degradation curves (mean ± 1 SD [error bars]). Spectrophotometry measurements were taken for Group 2 and Group 4 bottles. (A) This depicts the individual degradation curves for bottles 4, 5, and 6 (Group 2) over 5 wk. All time points were performed in triplicate. (B) This depicts the individual degradation curves for bottles 10, 11, and 12 (Group 4) over 6 wk. All time points were performed in triplicate. (C) Initial FAC levels for Group 2 were 4880 mg/L (SD = 23) and 4950 mg/L (SD = 12) for Group 4. FAC concentration decreased slightly with time for all bottles, to a final concentration of 4600 mg/L (SD = 21) for Group 2 (at week 5) and 4630 mg/L (SD = 31) for Group 4 (at week 6). Mean FAC loss from baseline levels was slightly higher in the not sprayed daily (Group 4) bottles (6% for Group 2 at week 5 compared with 7% for Group 4 at week 6), although this difference was not statistically significant. Note that daily spraying completely depleted the bottle contents of Group 2 by week 5.

pleted of diluted solution by 5 wk, suggesting that bottles can be refilled with fresh solution when empty without any loss of disinfection effectiveness in practice. This finding showed that laboratory animal programs can reduce labor and reagent costs associated with daily sodium hypochlorite solution preparation without compromising animal or technician safety. Our data also showed that actual concentrations of commercial bleach concentrates may be significantly lower than what is reported on consumer labels. This was also noted in another study that reported variability in advertised and measured concentrations of commercial bleach samples from different countries.[12] These findings strongly suggest that laboratory animal programs should empirically test the starting concentrations of purchased commercial bleach brands to develop dilution protocols that yield the desired 5000 mg/L disinfection concentration for use in animal spaces.

Prior studies show that extrinsic factors, including light, temperature, and air in bottle headspace, are important predictors of sodium hypochlorite solution stability.[1,2,5,6,8-13,15-17] We found that these factors remain reasonably stable in OHSU DCM animal housing spaces. We acknowledge that natural fluctuations in environmental parameters can be expected in any representative animal care and use program. Temperature was largely stable, apart from a 7-d period during which room temperature dropped to 18.9 °C, a 0.5 °C dip from the room minimum, possibly due to the absence of major thermal output (active racks with live animals) in the study room. Relative humidity levels, while not previously reported to affect FAC degradation, were out of the intended range for the majority of our experiment. We tracked temperature and relative humidity by using a thermo-humidity meter at the level of the bottles. These measures are highly dependent on placement of the meter within the room. Furthermore, relative humidity in animal rooms is commonly measured at the level of the building ducts. Light intensity, reported as lux, was within the range prescribed by the *Guide for the Care and Use of Laboratory Animals*, which recommend that empty rooms not exceed 400 lx approximately 1 m from the ground.[7] Conditions in this experiment represented the most extreme light conditions likely in an animal housing room. Prior studies found that light quality and quantity influence bleach stability.[2,5,8,9,11,16,17] Most, if not all, rodent housing rooms are completely devoid of natural light, so the compact fluorescent light tested in this experiment is most relevant to practice. Compact fluorescent bulbs emit a small amount of UVA, UVB, and infrared radiation, which we hypothesized might speed FAC degradation.[19] However, we found minimal decreases in FAC over the course of the study, such that environmental parameters reported here did not appreciably affect FAC concentrations. These FAC results may not be generalizable to laboratory animal spaces with less tightly controlled environmental parameters.

Prior studies also found that intrinsic factors, including pH and baseline FAC concentrations, contribute to bleach stability. Commercial bleach is commonly manufactured to a final pH greater than 11 because sodium hypochlorite solutions are more stable at higher pH.[5,16] Dilution of sodium hypochlorite with water lowers pH, which may speed solution degradation. Degradation increases at pH levels between 11 and 7 and increases precipitously at pH < 7.[1,5] Here, we found that the current OHSU DCM protocol yields a dilute sodium hypochlorite solution of pH approximately 11, and that this pH was relatively stable throughout the experiment, indicating that common practice and conditions will produce and maintain solutions of reasonable stability. Initial FAC concentrations can also affect solution

degradation. Solutions with relatively higher initial FAC concentrations generally degrade more quickly than solutions with lower starting FAC.[2,8,17] We found that the OHSU DCM protocol (adjusted for empirically measured commercial bleach concentration) yields dilute bleach solutions that vary minimally in starting FAC concentrations and that this minimal variance has negligible effects on solution degradation rate.

Measurement of FAC by spectrophotometry may not be feasible for all laboratory animal programs. Therefore, we tested the effectiveness of inexpensive and readily available colorimetric strips for evaluation of FAC concentrations. Colorimetric strips have lower resolution than spectrophotometry and can therefore not detect small fluctuations in FAC concentration. However, in this study, we found that fluctuations in FAC in dilute sodium hypochlorite solutions over 5 to 6 wk were not detectable on colorimetric strips; all bottles showed concentrations at 5000 mg/L, as measured by the strips. However, the FAC fluctuations that occurred in this study were minor, and this experiment spanned the full length of the likely "lifespan" of a bottle of solution in regular use, suggesting that colorimetric strips are a useful tool for a quick determination of adequate disinfection capacity of a given dilute solution. These strips may be a useful quality control tool for detecting the FAC of freshly prepared solutions, manufacturing changes in starting bleach concentrations, and errors in dilution. These events are not unlikely, as evidenced by the lower-than-expected starting concentration in the commercial bleach product used in this experiment. As mentioned in the materials and methods section, we altered the dilution equation from our SOP after determining that the FAC of the stock bleach bottle was lower than that stated on the bottle. Typically, the consumer does not know the lag in time between creation of the sodium hypochlorite solution and its distribution and use; increased distribution lag times may contribute to lower stock bleach concentrations. The CDC guidelines for disinfection and sterilization in healthcare settings and the instructions given by the bleach manufacturer recommend testing the solution using a quantitative method to fine tune dilutions to the specific concentration required.[12,18] However, the importance of using a 5000 mg/L sodium hypochlorite concentration may depend on disinfection use. For example, OHSU DCM's working solution (0.5%) is a much higher concentration than that required to kill a large majority of the microorganisms listed in the CDC guidelines (Table 1). This provides room for error. If a laboratory animal program has concerns about a specific microorganism that requires a high concentration of sodium hypochlorite, they may opt to use a higher concentration, or, alternatively, they may choose a different disinfection protocol that is more powerful (that is, vaporized hydrogen peroxide, chlorine dioxide).

Table 1. Microbicidal activity of chlorine and chlorine compounds. The table, according to the CDC guidelines on disinfection, lists the FAC concentration and contact time required to render the listed microorganisms inactive.

Microorganism	FAC Concentration Required for Disinfection (ppm)	Contact Time
Mycoplasma	25	15 s
Vegetative bacteria	<5	30 s
Mycobacterium tuberculosis	1000	<10 min
Bacillus atrophaeus spores	100	5 min
Mycotic agents	100	<1 h
Clostridium difficile	5000	≤10 min
25 different viruses	200	10 min
Candida	500	30 s
Staphyloccous aureus	100	<10 min
Salmonella cholerasuis	100	<10 min
Pseudomona aeruginosa	100	<10 min

FAC, Free Available Chlorine
ppm, parts per million

This experiment has several limitations. First, our results may be limited by our small sample size. We tested only 3 bottles per group, which is significantly fewer than the number of bottles in use in OHSU DCM. However, our sample size was consistent with other studies evaluating bleach stability, and we tested each bottle in triplicate to increase precision.[10,11] Second, the colorimetric strips we used have poor resolution at high concentrations, and we were unable to find alternative strips with better resolution. Manufacturers produce most colorimetric strips for FAC level estimation to determine the safety of drinking water treated with low levels of chlorine; they produce few colorimetric strips for use in high concentration solutions. In this experiment, all bottles tested with colorimetric strips were at the 5000 mg/L mark during the entire length of the experiment. However, these strips would not record less than a 50% loss in FAC; the next "color bar" records 2500 mg/L FAC. Therefore, these strips would only detect large errors or changes in manufacturing protocol. The colorimetric strips were also read by one unblinded individual. Results could have been strengthened by blinding the reader to the bottle conditions. However, this study was performed during the COVID-19 pandemic under modified operations at OHSU. Modified operations and campus research requirements dictated that only one individual at a time could work in a small, enclosed space. Third, we only tested 1 starting concentration of a dilute sodium hypochlorite solution (0.5% or 5000 mg/L) generated from a single commercial brand of bleach. Care should be taken when extrapolating to other bleach brands without empirical testing of FAC concentrations because starting concentration can influence bleach degradation. Fourth, we did not test for efficacy of solution disinfection through methods recognized by the Association of Official Analytical Chemists against common microorganisms; instead, we relied on published sodium hypochlorite concentrations previously reported to be effective. Last, we recognize that daily use in this study was an estimated average and that factors such as variability in how much people spray to clean surfaces and in how rooms are used contributes to how quickly bottles are emptied. Consideration for average room usage across facilities and animal care technician room task schedules will determine the exact rate at which sodium hypochlorite solutions are replaced; our data indicate that bottles can be prepared weekly, every other week, or possibly monthly. The findings of our study also support the need for a resource that shares detailed "true use" protocols for disinfectants to promote uniformity in protection.

The goal of this project was to provide practical guidance for the DCM on bleach stability. Here we show that in real-world conditions, 0.5% sodium hypochlorite solutions stored and used in a standard rodent housing room in opaque plastic bottles sprayed daily will maintain acceptable FAC concentrations for 5 to 6 wk, perhaps longer. Our results should directly inform practice changes that reduce technician time in preparing sodium hypochlorite bottles daily, reduce waste and help conserve resources, and reduce overall facility costs.

Acknowledgments

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