Drinking Water Acidification

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Laboratory animal drinking water is sometimes acidified continuously for disinfection. Edstrom Industries provides equipment for continuous acidification of automated watering systems (Central Proportioner) and for acidification of bottled water (Bottle Filling Proportioner / Station). This document provides answers to some common questions on the application of acidification for laboratory animals, such as:

- *"What pH level should the water be adjusted to?"*
- *"What type of acid should be used?"*
- *"What are the effects of acidified water on laboratory animals?"*

If you have any further questions or comments about acidification and drinking water quality, please contact Edstrom Industries at 800-558-5913.

What are the Benefits of Acidification?

Low pH water is bactericidal against Pseudomonas aeruginosa (an opportunistic pathogen common in water) and other Gram-negative bacteria. Acidification is used to prevent the spread of bacterial disease among laboratory animals through drinking water.

"Acidified water will not eliminate P. aeruginosa from infected mice nor necessarily prevent transfer of organisms from infected to non-infected cagemates via the fecal oral route. However, the elimination of the spread of P. aeruginosa and other bacteria through the water can be expected." (Small, 1983).

What pH is Recommended for Animal Drinking Water Disinfection?

As a general guideline, drinking water should be acidified to a pH of 2.5 to 3.0. One basis for this pH range is a study by Tanner and Samantha (1992) that concluded "solutions with a pH of 3 or lower had a bactericidal effect within 60 seconds against Pseudomonas aeruginosa and other Gram-negative eubacteria."

Some earlier studies on acidified water and animals used a pH as low as 2.0. However, because acidified water is quite corrosive, the pH should not be lower than necessary.

What Type of Acid Should be Used to Lower Drinking Water pH?

Hydrochloric acid (HCl) is most often used. Hermann (1982) reported using Tetracycline to acidify drinking water. Tetracycline has the advantage of having direct antibiotic activity.

Hall (1980) tested the effects of both hydrochloric and sulfuric acid treatment of drinking water on mice. This study found that water treated with sulfuric acid caused more pronounced health effects in irradiated mice than did water treated with hydrochloric acid.

The effectiveness of other acids against *Pseudomonas aeruginosa* can be found in Morton (1983) and Tanner and Samantha (1992).

What Piping Materials Can be Used With Acidified Drinking Water?

Water acidified to pH 2.5 – 3.0 is corrosive and must be used only in a system (or in water bottles) totally constructed of corrosion-resistant material. Acceptable materials include 316 stainless steel and plastics. Do not use brass, copper, or a lesser grade (304) of stainless steel. Even type 316 stainless steel, which is quite resistant, will be attacked to some degree by acidified water.

Can Any Microbes Grow in Acidified Water?

Yes, some microorganisms can multiply in low-pH water and others can survive (but not multiply). "Filamentous fungi and actinomycetes (filamentous bacteria like Nocardia) can adapt and multiply in pH 2 water. Spore-forming bacilli can resist pH of 2; however, they cannot multiply under those conditions." (Meltzer, 1993). Tanner and Samantha (1992) found that solutions with a pH of 3 or lower are bactericidal against Pseudomonas and other Gram-negative bacteria within 60 seconds. This effect was not observed on Gram-positive bacteria and yeasts.

Three animal facilities have reported acid-resistant microorganisms to Edstrom Industries:

- The first facility to report an acid-resistant organism found a fungus and a “free living parasite” in water acidified to pH 2.8. No exact I.D. was given.
- The second facility found a “filamentous organism with spores” in acidified water taken from wall-mounted manifolds that were never flushed. They chlorine sanitized and flushed out the manifolds and this cleared up the microbial growth.
- The third facility found Penicillium fungi and Exophiala (another fungus) in acidified drinking water. The Penicillium was also present in the incoming water supply. They chlorine sanitized the automated watering system to remove the growth.

Automated watering systems that deliver acidified drinking water should be periodically (monthly, quarterly, or annually as required) sanitized and flushed with a 20 ppm chlorine solution to kill acid-resistant microbes. Acid-resistant microorganisms aren’t usually a concern with bottled water since the bottles are already washed and sanitized regularly.

Caution

In an automated watering system, do not acidify chlorinated water below pH 5.0. At lower pH, chlorine will be present as dissolved chlorine gas (Cl_2) which may cause swelling of the silicon O-ring in animal drinking valves.

Where and When should pH be Measured?

Unlike chlorine in water, the pH of acidified water is relatively stable, so samples from various points in an automated watering system should have approximately the same pH. Initially, you will want to verify that samples taken from the farthest ends of an automated watering system have the same pH as samples from the Proportioner outlet. Daily sampling from the Proportioner outlet is recommended to ensure the acidified water is in the acceptable pH range. Or, an in-line pH analyzer can be installed to automatically monitor the treated water’s pH.

Sample holding time

For pH measurements, test a water sample immediately. Do not store samples to be analyzed for pH.

What are the Health Effects of Acidified Drinking Water?

Health effects on humans

In the pH range of 2.5 to 3.0, there is no immediate direct effect of pH in health of humans. Carbonated beverages have pH values between 2.0 to 4.0. The same can be said for foods (ex. apples 2.9 to 3.3) generally on the acid side of the scale. (DeZuane, 1990)

Health effects on laboratory animals

Several papers have been written on the effects of acidified water on mice. Please refer to the referenced papers for more details.

- **Les (1968)**: This study of the effect of acidified-chlorinated water (pH 2.5 and 10 ppm chlorine) on reproduction in C3H/HeJ and C57BL/6J mice during a period of 6 months indicated no detrimental effect of the water treatment.

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- **Hall, White, and Lang (1980)**: In this study, water acidified with either hydrochloric or sulfuric acid to pH levels of 2.0 or 2.5 was given for 6 weeks to normal and immuno-suppressed male, random-bred mice. The only significant changes in both normal and immunosuppressed mice were declines in weight gain and water consumption when the pH was 2.0. Water consumption wasn't effected when the pH was 2.5. Some of the observed changes were greater in the immuno-suppressed animals. When sulfuric-acidified water at pH 2.0 was consumed, the number of bacterial species isolated from the terminal ileum was reduced.
- **Hermann, White, and Lang (1982)**: In this study, water treated with hydrochloric acid, tetracycline, or sodium hypochlorite was given to mice for 120 days. Mice drinking water acidified to pH 2.0 had lower spleen weights and reduced spleen-to-body-weight ratios, which may reflect a slower growth rate. Overall, consumption of acidified drinking water did not appear to significantly affect the in vivo immune function of mice. They concluded that "these additives do not appear to be a serious health hazard to laboratory animals, but they do represent potential experimental variables."
- **Tober-Meyer, Bieniek, and Kupke (1981)**: The reaction of rats and rabbits to long-term acidified drinking water (pH 2.3 to 2.5) was observed over a 7-month period. The following parameters were studied: growth curves initiated at weaning, haematology, blood glucose, total serum protein, creatinine, electrolytes, and serum enzymes chosen to monitor the function of major organ systems. The results indicated that in rats and rabbits, the acidification of drinking water with hydro-

chloric acid to pH 2.3 to 2.5 did not have any adverse effect on the parameters examined.

Based on the current evidence, it is unlikely that acidified water represents a serious health hazard to laboratory animals at a pH of 2.5 to 3.0, but it should be evaluated as an environmental variable.

What is the Allowable pH Range for Public Water Supplies?

The minimum and maximum allowable pH range for potability is 6.5 to 8.5. This range is included in the secondary drinking water parameters of the Safe Drinking Water Act. Secondary maximum contaminant levels are federally non-enforceable and establish limits for contaminants in drinking water that may affect the aesthetic qualities and the public's acceptance of drinking water. Unlike primary drinking water parameters, "secondary" means not directly related to health.

The lower limit for pH is set to minimize corrosivity of water in municipal and residential distribution piping. DeZuane (1990) identified some problems caused by corrosivity:

- Contaminants like lead and cadmium can be picked up by corrosive water flowing through lead and galvanized pipe.
- Similarly, copper, iron, and zinc levels can increase when corrosive water is used in copper, galvanized, and unlined ductile and cast iron pipes.
- Concrete or asbestos-cement pipes are subject to corrosion at low pH that can release asbestos fibers into drinking water.
- Microorganisms may find protection in corrosive products, a common problem in municipal distribution systems of corrosive water.

The reason for establishing a lower pH limit does not apply in a laboratory animal automated drinking water designed to deliver acidified or purified water because all wetted materials are corrosion-resistant stainless steels or plastics.

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