

Chlorination of Drinking Water

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Laboratory animal drinking water is sometimes chlorinated continuously for disinfection. Other times, untreated drinking water is provided, but the automated watering system is periodically chlorine sanitized. Edstrom Industries provides equipment for either continuous chlorination (Central Proportioner, Chlorine Injection Stations) or periodic sanitization (Chlori-flush Stations, Portable Sanitizers)

This document answers some common questions on the application of chlorination for laboratory animals, such as:

- “What chlorine concentration should be used?”
- “What are the health effects of chlorine on laboratory animals?”
- “What are the byproducts of chlorine disinfection?”

If you have any further questions or concerns about chlorination or drinking water quality, contact Edstrom Industries at 800-558-5913.

What are the Benefits of Chlorination?

Chlorine, the most common disinfectant in the US, is effective in killing most pathogenic bacteria and viruses. Municipal potable water supplies are usually chlorinated to provide a residual concentration of 0.5 to 2.0 ppm. Chlorine is *not* effective in killing certain protozoans like cryptosporidium, however.

Why Add Chlorine to Animal Drinking Water?

If most tap water supplies are already chlorinated, why add more chlorine to animal drinking water? Additional chlorine is sometimes added for the following reasons:

To replace chlorine lost by dissipation in the municipal piping system

Oftentimes, chlorine in tap water has dissipated and is no longer present by the time it reaches the automated watering system. Low levels of chlorine are added back into the water in order to maintain low bacteria levels in the animal drinking water.

To combat chlorine-resistant microbes

To kill certain bacteria, such as *pseudomonas aeruginosa*, which are resistant to lower concentrations of chlorine, higher chlorine concentrations are needed. One pharmaceutical research facility determined through testing that they needed to chlorinate RO water to above 2 ppm free-chlorine to provide *pseudomonas*-free water.

To minimize biofilm

Continuous presence of chlorine in an automated watering system will minimize biofilm.

To ensure adequate disinfection when pH is high

Free chlorine is most effective at a pH of 5 to 7, where HOCl is the predominant form. The effectiveness declines with increased pH. Higher chlorine concentrations may be required to ensure adequate disinfection when the pH of water is high. Bauman (1962) recommends maintaining a chlorine residual of 5 ppm of free-chlorine to ensure the destruction of pathogenic bacteria and viruses for short contact times (5 to 10 minutes) and a pH of 7.0 to 8.5.

What Chlorine Level is Recommended for Continuous Disinfection?

As a general guideline, drinking water should contain 2- to 3-ppm free-chlorine *throughout* an automated watering system. This recommendation is based primarily on three factors: a survey by Edstrom Industries, an article that appeared in *Lab Animal* magazine, and unpublished verbal communication between researchers and Edstrom Industries staff.

Edstrom Industries microbiological survey

In this survey of nine animal facilities, a range of chlorine concentrations varying from 0.15 ppm to 3 ppm met microbial quality goals (Edstrom Industries, 1996).

***Lab Animal* magazine article: "Control of pseudomonas aeruginosa infection in mice by chlorine treatment of drinking water"**

In research on chlorine treatment for the control of pseudomonas aeruginosa infection in mice, a study concluded that the chlorine dose in bottled water should be adjusted so that the concentration does not fall below 2 ppm (Homberger et al, 1993).

Unpublished verbal communication

One pharmaceutical research facility has determined through their own testing that they need to maintain a minimum chlorine residual of 2 ppm free-chlorine in order to obtain low counts of chlorine-resistant microorganisms like pseudomonas aeruginosa.

Again, this 2- to 3-ppm free-chlorine recommendation is a general guideline or starting point. As a final check for adequate chlorination, test the microbial quality of water samples (see "Where and When Should Chlorine be Measured?" on page 5). Retest periodically to insure that drinking water disinfection is being maintained.

What Chlorine Level is Recommended for Periodic Sanitization?

Edstrom Industries recommends sanitization with 20-ppm chlorine for 30 to 60 minutes. From our experience, corrosion of 316 stainless steel has not been a problem at this dosage. Repeated sanitization at higher concentrations can cause corrosion of stainless steel drinking valves, manifolds, and room distribution piping in an automated watering system.

Is more chlorine better?

Although studies on the effects of chlorinated water have not found health problems in animals at elevated levels (see page 5 for information on health effects), it is sensible to use the smallest chlorine dosage that will provide adequate disinfection. Limiting the chlorine dosage will have the following benefits:

1. Minimize any potential effects on animal health. In the October 1996 Drinking Water Regulations and Health Advisories, the US Environmental Protection Agency (EPA) proposed a maximum contaminant level of 4 ppm for chlorine.
2. Minimize corrosion and material degradation. Excessive chlorine can corrode even stainless steel. It is also a problem with many elastomeric materials commonly used as seals, diaphragms, and o-rings in plumbing systems. When it comes to material compatibility, less chlorine is better.

Where and When Should Chlorine be Measured?

As a worst case (where the concentration will be the lowest), **measure the chlorine at points farthest from the incoming supply water right before flushing.** Take samples from the last interconnect quick disconnect or rack manifold drain valve in the room farthest from the proportioner. For chlorine measurements, do not flush the sample point before collecting a sample and test the sample immediately. At the same time, measure chlorine at the proportioner outlet and compare the concentrations. You may find that the proportioner outlet may need to be set to a higher concentration to compensate for chlorine dissipation in the automated watering system. For example, a chlorine level of 5 ppm at the proportioner may be required to provide 2- to 3-ppm free-chlorine throughout the piping system.

Sample holding time

For chlorine measurements, test the sample immediately. Chlorine in water is not stable and the concentration will decrease rapidly. Exposure to sunlight or other strong light or agitation will accelerate the reduction of chlorine. Therefore, start chlorine determinations immediately after sampling, avoiding excessive light and agitation. Do not store samples to be analyzed for chlorine.

What Causes Chlorine Dissipation in an Automated Watering System?

Chlorine may be used up reacting with organic matter and other oxidizable contaminants in the supply water and within the piping distribution system. The stability of chlorine will depend on the quality of your water supply and on the size and condition of the automated watering system. The chlorine demand of water is defined as the difference between the amount of the chlorine injected into the water and the amount of chlorine remaining at the end of a specified contact period, typically 20 minutes. Because it contains less chemical contaminants, the chlorine demand of reverse osmosis (RO) purified water is less than that of tap water. Thus, less chlorine needs to be injected into purified water to maintain the required residual concentration. An older automated watering system with accumulated biofilm inside the piping will have a higher chlorine demand than newly installed pipe and will require more chlorine injection, at least initially.

What are the Health Effects of Chlorine in Drinking Water?

Several papers have been written on the effects of chlorinated water on mice. All of these studies used chlorine concentrations at or above 10 ppm, which is higher than the chlorine level usually required in drinking water to control bacteria. Please refer to the referenced papers below for more details.

Blabaum and Nichols (1956)

In this first controlled study of chlorine in drinking water, these mice were provided with concentrations of chlorine in their drinking water of 100 and 200 ppm (with a pH of 5.9 to 6.5) for up to 50 days. "It was reported that there was no effect on body weight or gross histology. However, the conduct of the study appeared to be quite casual and data were not presented in a way that allows independent evaluation of the results." (Bull and Kopfler, 1991).

Les (1968)

This study of the effect of acidified-chlorinated water (with a pH of 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.

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 (Cl₂) which may cause swelling of the silicon seals in animal drinking valves.

Fidler (1977)

This study found a decrease in the number of peritoneal macrophages in mice only when the chlorine level was raised to 25 to 30 ppm. No adverse effect was observed when the chlorine level was 12 to 16 ppm.

Hermann (1982)

The purpose of this study was to determine if chronic ingestion of drinking water containing 30-ppm chlorine could change the *in vivo* immune responses in mice. Overall, no significant effect was observed.

DeZuane (1990)

DeZuane states that chlorine in water is fatal at 1,000 ppm, but that “consumption of drinking water containing 50 mg/L of chlorine has produced no adverse effect.”

Bull and Kopfler (1991)

In a study funded by the American Water Works Association, Bull and Kopfler provided the following review of toxicological effects that have been identified with chlorine in drinking water. Here is an excerpt from this study:

Administration of chlorine to rats and mice has failed to produce any specific toxic responses at concentrations well in excess of 250 mg/l (Druckrey 1968; Furukawa et al. 1980; Hasegawa et al. 1986; Kurokawa et al. 1986b). There was an indication of depressed weight gain at high concentrations, but this was related to decreased water consumption, reflecting aversion to the water at these concentrations. A more recent study, sponsored by the National Toxicology Program (NTP) (Southern Research Institute 1988a, 1988b), observed kidney changes with chlorine at 275 mg/L in drinking water, with no effect observed at 140 mg/L. These results have not yet been confirmed by the NTP's Pathology Working Group and must be considered tentative. One study indicated that relatively low doses of sodium hypochlorite induced sperm head abnormalities in mice (Meier et al. 1985b). However, there has been no association of this effect with other indicators of genetic or reproductive damage (Meier et al. 1985b; Carlton et al. 1986). A large number of epidemiology studies have noted an association between the use of chlorine as a disinfectant and increased risks of cancer (NAS 1987). The only study performed

with sufficient sophistication and power is the Cantor et al. (1987) study that associated bladder cancer with chlorinated surface waters versus nonchlorinated groundwaters in the United States. However, the focus of this and other studies was too narrow in terms of measures of water quality to (1) generalize results to all chlorinated waters or (2) identify the causative variables (e.g., distinguish between chlorine residual or its by-products).

Based on the current evidence, it is unlikely that chlorine itself represents a health hazard to laboratory animals at the concentrations (less than 10 ppm) typically found in automated watering systems. A greater chance for health effects may be caused by chlorination by-products.

What are Chlorination By-Products?

One complication that arises in the control of microorganisms is the formation of by-products during the disinfection process. Chlorination by-products are chemicals that result from the reaction of chlorine with organic substances in water. Trihalomethanes (THMs) refer to one class of disinfection by-products found in nearly every chlorinated public water supply. The highest levels are found in surface water supplies that have higher levels of naturally occurring organic contaminants. Table 1 lists some common chlorination by-products. The most prevalent is chloroform (trichloromethane), a THM which is carcinogenic to rats and mice.

By-product	Concentrations found in chlorinated waters
Chloroform	Range 0.7 - 540 µg/L, Mean 26.4 µg/L
Bromodichloromethane	Range 1.9 - 183 µg/L, Mean 9.1 µg/L
Chlorodibromomethane	Range 0.4 - 280 µg/L, Mean 5.7 µg/L
Bromoform	Range 0.4 - 280 µg/L, Mean 5.7 µg/L
Chloroacetic Acid	Range <1 - 5 µg/L
Dichloroacetic Acid	Range 12 - 79 µg/L, Mean 47 µg/L
Trichloroacetic Acid	Range 4 - 103 µg/L, Mean 38 µg/L
Dichloroacetonitrile	Range 1.9 - 24 µg/L, Mean 2 µg/L

Table 1. Common chlorination by-products.

It is important to note that chlorination by-products are already likely to be present in the chlorinated municipal tap water supplied to your animal facility. Injecting additional chlorine into animal drinking water may or may not increase the amount of disinfection by-products. The principle method for removal of chlorination by-products like chloroform is by activated carbon adsorption. Since carbon filters also remove chlorine, they should be placed before chlorine injection.

If a water supply contains organic contaminants or precursors that react with chlorine, the formation of disinfection by-products can be avoided by removing the organics. Reverse osmosis is effective in removing the larger organic precursors, but is not very effective in removing smaller molecules of chloroform and other trihalomethanes. Compared to tap water, fewer new chlorination by-products are created when chlorinating purified RO water.

What are the Health Effects of Chlorine By-Products?

Disinfection by-products have been linked to adverse health effects, including cancer, in laboratory animals. Table 2 summarizes the health effects of some chlorination by-products.

By-product	Health effects
Chloroform	Animal carcinogen that can induce liver tumors in mice and kidney tumors in rats.
Bromodichloromethane	Produces liver and kidney damage in both mice and rats. Carcinogenic in mice and rats, producing renal, liver, and intestinal tumors.
Chlorodibromomethane	Produces liver and kidney damage in both mice and rats. Induces tumors in the liver of mice.
Bromoform	Low incidence of intestinal tumors in rats.
Chloroacetic Acid	Neurologic effects in animals. No increased tumors.
Dichloroacetic Acid	Major toxicities are damage to the nervous system and liver. Induces liver tumors in mice.
Trichloroacetic Acid	Potent inducer of liver tumors in male mice.
Dichloroacetonitrile	No specific toxicological effects reported, only nonspecific effects on body weight and some organ weights and some reproductive effects.

Table 2. Health effects of various chlorination by-products.

Understand that these chemicals are tested in animals at much higher doses than would be expected in chlorinated municipal drinking water. In determining regulatory limits in drinking water, the EPA performs risk assessments using conservative safety factors. The current regulatory limit for total combined trihalomethanes in water is 0.1 mg/L (ppm). In the *1994 Proposed Rule for Disinfectants and Disinfection By-Products*, the total combined THMs cannot exceed 0.08 mg/L.

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