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Drinking Water Quality Standards

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Over the years, many animal facility managers have called Edstrom Industries to ask "What are the standards for laboratory animal drinking water?", "What bacterial level is acceptable?", and "What should we test water for?" They are looking for something more specific than the general statement in the 1996 revision of *The Guide for the Care and Use of Laboratory Animals*, which states:

> *"Ordinarily animals should have continuous access to fresh, potable, uncontaminated drinking water, according to their particular requirements. Water quality and the definition of potable water can vary with locality (Homberger and others 1993). Periodic monitoring for pH, hardness, and microbial or chemical contamination might be necessary to ensure that water quality is acceptable, particularly for use in studies in which normal components of water in a given locality can influence the results obtained. Water can be treated or purified to minimize or eliminate contamination when protocols require highly purified water. The selection of water treatments should be carefully considered because many forms of water treatment have the potential to cause physiologic alterations, changes in microflora, or effects on experimental results (Fidler 1977; Hall and others 1980; Hermann and others 1982; Homberger and others 1993). For example, chlorination of the water supply can be useful for some species but toxic to others (such as aquatic species).*

For a more specific water quality standard, we recommend the following:

1. **Meet Environmental Protection Agency (EPA) standards.** It makes sense to interpret "potable, uncontaminated" water as water meeting the EPA's Primary (health-related) Drinking Water Standards for human consumption.
2. **Set limits and test for contaminants of concern to your facility's research studies.** Examples: For toxicological studies, test for any interfering chemical contaminants in drinking water. For immune-compromised animals, set tighter limits on bacterial contaminants.
3. **Learn from limits set by other facilities and organizations.** Some animal facilities have set their own microbial water quality goals (see the *Microbiological Survey of Drinking Water Systems* – Edstrom document). To learn about bacterial quality levels achievable in piped water systems, refer to the United States Pharmacopeia (USP) standards for pharmaceutical water.
4. **Monitor the purification process if purified water is specified.** Water purification (usually by reverse osmosis) will remove contaminants from water and will provide a standardized water quality safeguarded against upsets in the local tap water supply. Monitor the purification process, usually by testing conductivity or percent rejection.

At the Charles River Symposium in 1980, Gordon Newell suggested that the laboratory animal industry should consider a cooperative effort "to establish water quality standards before any regulatory agency steps in and dictates water quality rules." This has yet to be done.

The purpose of this document is to recommend animal drinking water quality standards based on EPA standards, standards for pharmaceutical waters, and on what some of the more proactive facilities have done in their organizations. Edstrom Industries hopes this document will help facility managers in setting their own water quality standards. We welcome input and suggestions from the laboratory animal research community! If you have any suggestions or questions, please call Edstrom Industries at 1-800-558-5913 or send e-mail to lab@edstrom.com.

EPA Drinking Water Standards

Most facilities interpret the "potable, uncontaminated" water requirement for ordinary animals as water that meets, at a minimum, the EPA drinking water standards for human consumption. What are the EPA standards?

The EPA is responsible for the **National Primary Drinking Water Regulations**, which are health-related standards that establish the Maximum Contaminant Levels (MCLs). MCLs are the maximum permissible level of a contaminant in water delivered to users of a public water system. MCLs are enforceable under the Safe Drinking Water Act. The EPA has also set unenforceable Maximum Contaminant Level Goals (MCLGs) at levels where no known or anticipated adverse effects on health occur and that allow an adequate margin of safety. The enforceable MCL is set as close to the MCLG as reasonable, taking into consideration the costs and treatment techniques available to public water systems.

Health advisories provide information on contaminants that can cause human health effects and are known or anticipated to be in drinking water. Health advisories are guidance values based on non-cancer health effects for different durations of exposure (e.g., one-day, ten-day, longer-term, and lifetime).

National Secondary Drinking Water Standards

National Secondary Drinking Water Standards are unenforceable federal guidelines regarding taste, odor, color, and certain other aesthetic characteristics of water. The EPA recommends them to states as reasonable goals, but federal law does not require water systems to comply with them.

The EPA drinking water standards are listed in Table 1. For the most recent regulations, you can order a free copy of *Drinking Water Regulations and Health Advisories* from the safe drinking water hotline.

Safe Drinking Water Hotline
(800)-426-4791
Monday through Friday, 9:00 AM to 5:30 PM EST

USP (United States Pharmacopeia) Specifications

It is useful to learn about pharmaceutical water standards to see what can be applied to animal drinking water. This is especially true for bacterial contaminants. It is impossible to achieve absolutely sterile water in any piped water system, but automated watering systems can (with appropriate design and operation) achieve the quality of USP Purified Water and can approach USP Water for Injection.

What is the USP?

The United States Pharmacopeia Convention is a private, not-for-profit organization that sets standards for drugs, devices and diagnostics. It publishes two compendia (summary documents). The US Pharmacopeia (USP) contains standards for drug products. The National Formulary (NF) sets standards for drug excipients (inert substances used as carriers or dilutants).

Legal Status

The standards listed in the "monograph" section of the USP are legally enforceable by the FDA. The "General Information" section is not enforceable by the FDA.

USP Waters

The USP monograph lists two waters that are prepared in bulk form: **Purified Water (PW)**, often called **USP Purified Water** to distinguish it from other purified waters, and **Water for Injection (WFI)**.

USP Purified Water (PW)

Purified Water is described in the USP 23 monograph as follows:

> *"Purified Water is water obtained by distillation, ion-exchange treatment, reverse osmosis, or other suitable process. It is prepared from water complying with the regulations of the U.S. Environmental Protection Agency (EPA) with respect to drinking water. It contains no added substances."*

Microbial Quality

Regarding the bacteriological purity of PW, the monograph (legally enforceable section) states only that PW must comply with the EPA regulations for drinking water. The EPA regulations only specify limits for coliform bacteria. In the informational section of the USP 23, which deals with action guidelines for the microbial control of ingredient water, it says:

> *"A total microbial (aerobic) count that may be used for source drinking water is 500 colony-forming units (cfu) per mL. A general guideline for Purified Water may be 100 cfu/mL."*

These numbers for cfu/mL are only advisory guidelines that represent recommended alert/action limits, not reject levels. The informational section also suggests that the microbial action limits for PW should be based on the intended use of the water and the nature of the product being made. It recognizes that microbial limits for PW require being defined on a case-by-case basis.

USP 23 Supplement 5, effective since November 1996, specifies the method for total bacteria counts. It states "Heterotrophic Plate Count of a 1-mL sample, using Plate Count Agar at an incubation temperature of 30 to 35°C for a 48-hour period (minimum)." There is some controversy (Collentro 1996) because this method will underestimate "starved" bacteria in high-purity water.

Chemical Quality

Effective November 15, 1996, the former inorganic chemistry tests (for calcium, sulfate, chloride, ammonia, and carbon dioxide) were replaced with a three-stage conductivity test. The conductivity limit is pH-dependent but, for example, at pH 7.0, conductivity should be less than 5.8 microSiemen/cm (µS/cm). The former test for oxidizable substances was replaced with a Total Organic Carbon (TOC) limit of 0.05 mg/L. TOC is an indirect measure of organic molecules present in water measured as carbon. The new tests allow continuous in-line monitoring of water using instrumentation rather than lab work.

Water for Injection (WFI)

The USP 23 monograph states:

> *"Water for Injection (WFI) is water purified by distillation or reverse osmosis."*

WFI is produced by either distillation or 2-stage RO. It is usually stored and distributed hot (at 80°C) in order to meet microbial quality requirements.

Microbial Quality

The USP monograph makes no references to bacteria limits for WFI. It does not need to be sterile; however, the monograph specifies that WFI not contain more than 0.25 USP endotoxin units (EU) per mL. Endotoxins are a class of pyrogens that are components of the cell wall of Gram-negative bacteria (the most common type of bacteria in water). They are shed during bacterial cell growth and from dead bacteria. Indirectly, the water must be of a very high microbial quality in order to have a low endotoxin concentration. The USP informational section recommends an action limit of 10 cfu/100 mL. The recommended method of testing is membrane filtration of a 100-mL sample and plate count agar at an incubation temperature of 30 to 35°C for a 48-hour period.

Chemical Quality

The chemical purity requirements of WFI are the same as PW.

Recommendations for Lab Animal Drinking Water

Standards for Chemical Quality

1. **EPA Regulated Chemical Contaminants.** The requirement for "potable, uncontaminated" drinking water for "ordinary animals", as stated in the Guide, can be interpreted to mean that animal drinking water should, at a minimum, comply with the EPA's Primary (health-related) Drinking Water Regulations for human consumption. This is similar to the requirement that states USP Purified Water must be prepared with water complying with the EPA regulations. The EPA regulated contaminants are listed in Table 1.

   Recommendation: Water should meet EPA Primary Drinking Water Standards. Test for the list of regulated chemicals at your facility or obtain documentation from your local water utility. Animal facilities that test for these contaminants usually do so 1 to 4 times per year.

2. **pH.** The Guide specifically mentions pH as something that may necessitate periodic monitoring. The reason for setting a low limit is that low pH water is corrosive and can dissolve plumbing components. This is especially a concern when water contacts brass and copper piping systems where copper, zinc, and lead can dissolve into the animal's drinking water. Reasons for setting a high limit are that high pH can promote hardness scale precipitation (see number 3 "Hardness" below) and that chlorine disinfection is not as effective at high pH. See the Edstrom Industries document *Forms of Chlorine in Water (MI-4148)* for more information. Some facilities acidify animal drinking water to pH 2.5 to 3.0 in order to kill Pseudomonas and other common water bacteria.

   Recommended ranges for acceptable pH:

   - 6.5–8.5 for water that contacts brass and copper plumbing components
   - 2.5–8.5 for water in stainless steel and/or plastic piping systems

   Note: For most effective chlorine disinfection, pH should be below 7.0

3. **Hardness.** The reason for setting an upper limit on hardness is that hard water can cause calcium carbonate scale deposits in automated watering systems, which can lead to drinking valve leaks and other operational problems. According to the Water Quality Association, water is considered "hard" when the measured hardness exceeds 120 mg/L.

Just knowing the hardness level of water is not enough to predict if it will cause scaling problems. A better predictor is the Langelier Saturation Index (LSI). When the LSI (which is calculated from pH, total dissolved solids, calcium hardness, and alkalinity) is greater than zero, water will have a tendency to scale. See the Edstrom Industries document *Scale-forming Tendency of Water (MI-4170)* for more information.

Recommendation: LSI of water in automated watering systems should be less than zero.

4. **Total Dissolved Solids or Conductivity.** Total dissolved solids (TDS) and conductivity both indicate the total inorganic mineral content of drinking water. Either of these tests can be used to monitor the consistency of quality from water purification processes (such as reverse osmosis), which remove inorganic contaminants from water. The typical conductivity of reverse osmosis (RO) water ranges between 1-100 µS/cm, depending on the conductivity of the supply water. Usually, conductivity is measured with in-line sensors. For more information on monitoring water purifications, see *Monitoring an RO System (MI4154)*.

   Recommendation: When water is purified by reverse osmosis, deionization, or distillation, the purification process should be monitored by measuring the TDS or conductivity of the product water. Maximum limits should be set based on the supply water quality and the specified performance of the purification process.

5. **Disinfectants.** An automated drinking water system may contain residual disinfectants from the public drinking water supply or additional disinfectants may be injected into animal drinking water to control bacterial growth. The EPA has proposed/tentative maximum contaminants levels for these common disinfectants:

Chloramine	proposed MCL = 4 mg/l
Chlorine	proposed MCL = 4 mg/l
Chlorine dioxide	tentative MCL = 0.8 mg/l

   Recommendation: Drinking water disinfectants should be measured periodically to document the level in animal drinking water. If additional disinfectant is added at the animal facility, the concentration is typically measured 1-7 times per week, both at the point of injection and at the farthest point downstream in the automated watering system's piping.

6. **Other.** Test animal drinking water for any other contaminants that might interfere with research protocols at your facility.

Standards for Microbial Quality

In order to establish microbial quality guidelines for animal drinking water, it is useful to understand the guidelines and regulations for EPA drinking water and for USP PW and WFI.

1. **Specify Treatment Techniques**. EPA regulations specify treatment techniques (filtration, disinfection, etc) in lieu of specific maximum contaminant limits for most microbial contaminants in drinking water. Because of the complexity and cost of testing for waterborne microorganisms, routine examination of water for pathogens is not feasible. Even when specific pathogens are examined, a negative result may be due to the inadequacy of the testing method. Or, it may indicate a safe water quality at that moment when the water was sampled, but it does not guarantee that safe water can be expected one hour before or after testing.

   If this type of guideline were adapted for laboratory animal drinking water, a facility may, for example, specify reverse osmosis water containing a minimum concentration of

disinfectant in lieu of specifying maximum concentrations of bacteria, viruses, and pathogenic protozoa.

2. **Set Limits for Certain Specific Microorganisms.** The EPA's MCL for coliform bacteria is less than one per 100 mL and the unenforceable MCLG for Giardia lamblia, Legionella, and viruses is zero.

3. **Set Limits Based on Use of Water.** USP Purified Water guidelines suggest an action/alert limit for total microbial counts at 100 colony-forming-units/mL, but it also recognizes that microbial limits should be defined on a case-by-case basis. Just as the bacteria counts limits for USP PW should be based on the intended use of the water, microbial limits for animal drinking water may need to be based on the requirements for the animals or the study. Perhaps there needs to be separate recommendations for different animal and study types.

4. **Do Not Expect Absolutely Sterile Water**. It is impossible for the water delivered through any piping system to be totally free of bacteria. To understand how bacteria attach and grow within even purified water systems, call Edstrom Industries and ask for a copy of the paper on Biofilms (Dreeszen, 1997), or read it on our Web site: http://www.edstrom.com/Resources.cfm?doc_id=23.

   Even the highest quality pharmaceutical water is not expected to be absolutely sterile. Although USP WFI is stored and distributed hot at 80°C, the recommended action limit is 10 cfu/100 mL (not "zero"). Very low bacteria counts are possible in ambient temperature automated watering systems, but drinking water will not be sterile.

Recommended Guidelines

Here are some recommended guidelines for laboratory animal drinking water:

1. **Coliform Bacteria.** The coliform test is a reliable indicator of the possible presence of fecal contamination and is, consequently, correlated with pathogens. The EPA MCL is less than one coliform per 100 mL. Many animal facilities periodically test animal drinking water for coliforms and use this limit. (Refer to Microbiological Survey of Automated Watering Systems, Dreeszen, 1996.)

   Recommendation: Animal drinking water should be the same as EPA potable water. (<1 coliform/100mL).

2. **Total Plate Counts.** The Heterotrophic Plate Count (HPC) test, also called "total count"or "plate count", provides an estimate of the total number of bacteria in a sample that will develop into colonies during a period of incubation in a nutrient. This test detects a broad group of bacteria including nonpathogens, pathogens, and opportunistic pathogens, but it does not pretend to report all of the bacteria in the water sample examined. HPC may be an indicator of poor general biological quality of drinking water.

   Health agencies like the EPA and World Health Organization (WHO) "have avoided setting standards for plate counts, possibly for lack of pathogenicity and great variation in density encountered (DeZuane, 1990)." A recommended MCL for human drinking water has not yet been proposed, but the EPA does recognize the water quality deterioration implied by high plate counts. The upper limit for potable water is usually 500 colony-forming units or cfu/mL. One reference (DeZuane 1990) says water with counts under 100 cfu/mL should be considered "potable" and values 100-500/mL "questionable".

   USP PW has an unenforceable action guideline of 100 cfu/mL. Because plate counts under 100/mL should be easily obtained in chlorinated water, this could be a general action/alert level for most animal drinking water. If counts are generally under 100/mL,

normal fluctuation should keep counts under 500/mL. Many animal facilities have set their own limits for HPC bacteria, which range from <1 cfu/mL to <1000 cfu/mL. (Microbiological Survey, Dreeszen, 1996)

Recommendation: For ordinary animals, a total bacteria count alert/action level 100 cfu/mL is achievable in automated drinking water systems and will result in good overall bacterial quality. Lower levels may be desired for immunocompromised animals or for special animal models.

3. **Pseudomonas aeruginosa.** Pseudomonas aeruginosa is a common water bacteria and is an opportunistic pathogen, which can infect immune-compromised animals. In Edstrom Industries' *Microbiological Survey* (Dreeszen, 1996), facilities set goals for Pseudomonas anywhere from <1/100mL to <1000/mL. This is one standard that animal facilities think is important, but the recommended level may depend on the type of animals.

   Recommendation: Facilities housing immunocompromised animals should monitor and set limits for Pseudomonas aeruginosa. A common limit set by several facilities is <1 cfu/mL.

4. **Viruses and Pathogenic Protozoans.**

   Although the EPA's MCLG for viruses and Giardia is zero, detection of viruses and protozoans (which also includes Cryptosporidium) is difficult and not technically feasible for routine analysis of human drinking water.

   Recommendation: If viruses or pathogenic protozoans are of concern, perhaps an effective water treatment technique such as reverse osmosis or ozonation could be specified instead of specifying a contaminant limit.

Comparison of Water Quality Standards

Table 1 summarizes and compares EPA drinking water standards, USP pharmaceutical water standards, and suggested standards for laboratory animal drinking water. The asterisk * indicates that the limit is the same as the EPA Primary Standard MCL. Other symbols and abbreviations are defined at the end of the table.

Table 1. Water Quality Standards (mg/l unless other units given).

Contaminant	EPA Standards (as of 10/96)			USP 23 (as of 11/96)		Animal Drinking Water
	Primary MCL (1)	MCLG (2)	Secondary MCL (3)	Purified Water	WFI	
Organic Contaminants						
Adipate (diethylhexyl)	0.4	0.4		*	*	*
Alachor	0.002	zero		*	*	*
Aldicarb	0.007	0.007		*	*	*
Aldicarb sulfone	0.007	0.007		*	*	*
Aldicarb sulfoxide	0.007	0.007		*	*	*
Atrazine	0.003	0.003		*	*	*
Benzene	0.005	zero		*	*	*
Benzo(a)pyrene (PAH)	0.0002	zero		*	*	*
Carbofuran	0.04	0.04		*	*	*
Carbon Tetrachloride	0.005	zero		*	*	*
Chlordane	0.002	zero		*	*	*
2,4-D	0.07	0.07		*	*	*
Dibromochloropropane (DBCP)	0.0002	zero		*	*	*
p-Dichlorobenzene	0.075	0.075		*	*	*
o-, m-Dichlorobenzene	0.6	0.6		*	*	*
1,2-Dichloroethane	0.005	zero		*	*	*
1,1-Dichloroethyene	0.007	0.007		*	*	*
cis-1,2-Dichloroethylene	0.07	0.07		*	*	*
trans-1,2-Dichloroethylene	0.1	0.1		*	*	*
1,2-Dichloropropane	0.005	zero		*	*	*
Di(2-ethylhexyl)phthalate (PAE)	0.006	zero		*	*	*
Dinoseb	0.007	0.007		*	*	*
Diquat	0.02	0.02		*	*	*
Endothall	0.1	0.1		*	*	*
Endrin	0.002	0.002		*	*	*
Ethylbenzene	0.7	0.7		*	*	*
Ethylene dibromide	0.00005	zero		*	*	*
Glyphosate	0.7	0.7		*	*	*
Heptachlor	0.0004	zero		*	*	*
Heptachlor epoxide	0.0002	zero		*	*	*
Hexachlorobenzene	0.001	zero		*	*	*
Hexachlorocyclopentadiene	0.05	0.05		*	*	*
Lindane	0.0002	0.0002		*	*	*
Methoxychlor	0.04	0.04		*	*	*
Monochlorobenzene	0.1	0.1		*	*	*
Oxamyl (Vydate)	0.2	0.2		*	*	*
Pentachlorophenol	0.001	zero		*	*	*
Picloram	0.5	0.5		*	*	*
Polychlorinated biphenyls (PCBs)	0.0005	zero		*	*	*
Simazine	0.004	0.004		*	*	*
Styrene	0.1	0.1		*	*	*
Tetrachloroethylene	0.005	zero		*	*	*
Toluene	1.0	1.0		*	*	*
Toxaphene	0.003	zero		*	*	*
2,4,5-TP Silvex	0.05	0.05		*	*	*
Trichlorobenzene (1,2,4-)	0.07	0.07		*	*	*
1,1,1-Trichloroethane	0.2	0.2		*	*	*
1,1,2-Trichloroethane	0.005	0.003		*	*	*
Trichloroethylene	0.005	zero		*	*	*
Vinyl chloride	0.002	zero		*	*	*
Xylenes	10	10		*	*	*
Total trihalomethanes	0.10	zero		*	*	*
Total Organic Carbon (TOC)				0.05	0.05	*

Contaminant	EPA Standards (as of 10/96)			USP 23 (as of 11/96)		Animal Drinking Water
	Primary MCL (1)	MCLG (2)	Secondary MCL (3)	Purified Water	WFI	
Inorganic Contaminants						
Antimony	0.006	0.006		*	*	*
Arsenic	0.05	-		*	*	*
Asbestos	7 MFL	7 MFL		*	*	*
Barium	2	2		*	*	*
Beryllium	0.004	0.004		*	*	*
Cadmium	0.005	0.005		*	*	*
Chromium	0.1	0.1		*	*	*
Copper	1.3	1.3	1.0	*	*	*
Cyanide	0.2	0.2		*	*	*
Fluoride	4.0	4	2.0	*	*	*
Lead	0.015	zero		*	*	*
Mercury	0.002	0.002		*	*	*
Nickel	0.1	0.1		*	*	*
Nitrate (as N)	10.0	10.0		*	*	*
Nitrite (as N)	1.0	1.0		*	*	*
Selenium	0.05	0.05		*	*	*
Thallium	0.002	0.0005		*	*	*
Aluminum			0.05 to 0.2			
Chloride			250	(4)	(4)	
Iron			0.3			
Manganese			0.05			
pH			6.5-8.5	5.7-7.0	5.7-7.0	6.5-8.5 (brass) 2.5-8.5 (ss/plastic)
Silver			0.1			
Sulfates			250	(4)	(4)	
Total dissolved solids (TDS)			500	(4)	(4)	
Zinc			5.0			
Ammonia				(4)	(4)	
Calcium				(4)	(4)	
Heavy Metals				(4)	(4)	
Conductivity				4.7-5.8 µS/cm (depending on pH)	4.7-5.8 µS/cm (depending on pH)	
Radionuclides						
Gross alpha particle activity	15 pCi/L	++		*	*	*
Beta particle and photon activity	4 mrem/yr	++		*	*	*
Radium 226 and 228 (total)	5 pCi/L	++		*	*	*
Microbiological Contaminants						
Coliforms (total)	<1/100 mL	zero		*	*	*
Giardia lamblia	TT	zero				TT
Heterotrophic Plate Count	TT	NA		100 cfu/mL**	10 cfu/100 mL**	100 cfu/mL **
Legionella	TT	zero				
Pseudomonas sp.						1 cfu/mL**
Pyrogens					0.25 EU/mL	
Turbidity	0.5-1.0 NTU	NA				*
Viruses	TT	zero				TT

Notes:

(1) National Primary Drinking Water Standards Maximum Contaminant Levels are federally enforceable.

(2) Maximum Contaminant Level Goals are not enforceable.

(3) National Secondary Drinking Water Standards are not federally enforceable. These limits are for contaminants that may affect the aesthetic qualities of water.

(4) These wet chemistry tests were replaced by a conductivity standard per UPS 23 Fifth Supplement effective 11/15/96.

* Assume same as EPA Primary Drinking Water Standard MCLs

** Action Guidelines, not enforceable limits, limits set on a case-by-case basis.

TT Treatment technique is specified, not the measured concentration

++ No final MCLG, but zero proposed in 1991

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