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Water Quality Standards

Helen Kelly

For years the impact of water contamination was the subject of movies, best sellers, editorials and speculation. Today, thanks to persistence and rigorous research, we know: what's in the water influences a living system's biochemistry and bio-behavioral response.

Still, water quality standards for lab animal research are spare. Consider Australia's *Code of Practice for the Care and Use of Animals for Scientific Purposes*[1] water quality standard: clean, fresh drinking water should be available at all times as suitable for the species. The water quality standard specified in the *Guide for the Care and Use of Laboratory Animals* is similarly straightforward: provide clean, potable water. That's all they and most other animal care guidelines prescribe and it is clear cut, yet some scientists say these simple statements raise a raft of complex questions. What do clean, fresh and potable mean in a research setting? Since water quality standards vary from locale to locale, what is the impact of difference on concurrent studies or ability to replicate a study? What are the interactions among new molecules and micronutrients in water that might inhibit or decrease uptake?

Furthermore, does water deemed suitable for human consumption necessarily meet research needs? Many scientists say the answer is decidedly no. In addition to the sometimes subtle and often conspicuous differences setting to setting, there is runoff residue that includes medicines, growth hormones, industrial waste, pesticides, and perilous organisms that mostly aren't regulated for humans and could dramatically influence research findings.

It would seem that for now, despite some downsides, the answer for lab animal research is to set a baseline by purifying the water. Professor Joseph L. Taraba, a University of Kentucky, USA specialist on groundwater quality and health[2], says that depleting water of needed nutrients could be the other side of the impure water coin, yet even so, for now we must purify. “Minimising error in testing health impacts in living systems is quite a challenge. There are so many feedback mechanisms in complex organisms, and so many micronutrients that can be important to any living organism's health; if it lacks something unknown it becomes an uncontrolled variable and may affect the quality of results. And, when it comes to water, there are so many differences area to area in water quality–differences such as presence of metals, calcium, phosphorous, cations and anions, trace organics—that might influence an organic system’s feedback mechanisms. Yet, despite all the unknowns, I suggest we should err on the side of caution. The practical way ahead right now is to purify the water as much as possible and hope that the food used in a testing protocol will make up for the nutritional discrepancies or deficiencies.”

So, are the guidelines adequate? Are clean, fresh, and potable as specific as one could reasonably be given the infinite variables for which anything more exact would have to account? And if that’s so, how might facility managers and investigators work together to standardise water at least for short term requirements? - HK

References

1. Australian Code of Practice
2. Professor Joseph L. Taraba is a University of Kentucky specialist in Biosystems and Agricultural Engineering for animal production impacts on the environment, surface, and groundwater quality impacts on human and animal health.

***Helen Kelly** is a Contributing Editor for ALN World™.*

Rethinking Global Water Quality Standards

http://www.alnmag.com/article/rethinking-global-water-quality-standards

Providing consistent and repeatable water quality will instill confidence that the water remained the same and within specification for the duration of your study.

Bob Lemken (ghostwritten by Helen Kelly)

The composition of water will influence research results. Regarding the potential effects of a variable water quality on the repeatability of a research project, I would offer the following comments and reference provided by Dr. Tim Martin, Director, Animal Care Program, St. Joseph’s Hospital and Medical Center, Phoenix, USA.

I feel it is imperative that our industry establish quality standards for drinking water. The only requirement at this time is for the “potable, uncontaminated drinking water” [from the Guide for the Care and Use of Laboratory Animals]. The problem with this statement is the variability in the mineral and chemical content from state to state, city to city, and institution to institution. This difference can directly affect the outcome or repeatability of research projects. An example of this can be found in an article from the Proceedings of the National Academy of Science USA (2003 Sep 16; 100(19): 11065-9) entitled “Trace amounts of copper in water induce amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease”. The level of copper present in drinking water affected the quality of the Alzheimer’s model. Some of the original work on this model was conducted at an AAALAC accredited facility where the rabbits received their water from an automated watering system. The source of the water was the municipal supply. The drinking water was routinely tested for impurities and bacterial contamination and the *results were always within the established [US] EPA standards. The animals developed plaques as expected. When the project was moved to another institution 25 miles away, the rabbits did not respond the same. They had developed fewer plaques. The only difference was the source of water. At the second institution, all the animals were given distilled bottled water.*

Both institutions adhere to the current standards for animal husbandry; however just one minimal difference in husbandry practice greatly affected the outcome of a research project.

WHAT IMPURE WATER CAN DO

To understand just how potent impure water can be, think about *cryptosporidium*. This is a resilient waterborne parasite that travels in a protozoan cyst. The cyst travels via warm-blooded animal feces into water sources and enters the animal host predominantly via ingestion. Once inside the host, the organism ensures survival by reproducing asexually and sexually before building itself two different types of floating mobile home: a thick-walled oocyst that gets excreted and a thin-walled oocyst that infects the body (Figure 1).

The parasite causes intestinal infection, revealing itself only when the host comes down with full blown diarrhea and stomach cramps between one and twelve days later.

The infection spreads like wildfire among animal populations—and in breeding facilities, tens of thousands may become infected. In municipalities, contaminated water sources are so interlinked, cryptosporidiosis is nearly impossible to contain. In one of many recent outbreaks in the UK and the US, 400,000 people in Milwaukee, USA became ill with cryptosporidium and 100 people died.

The real issues with cryptosporidiosis are treatment and removal. The organism resists traditional water purification chemicals such as chlorine, and older water treatment facilities aren’t set up to deal with stronger disinfectants. The process of reverse osmosis will remove cryptosporidium from water.

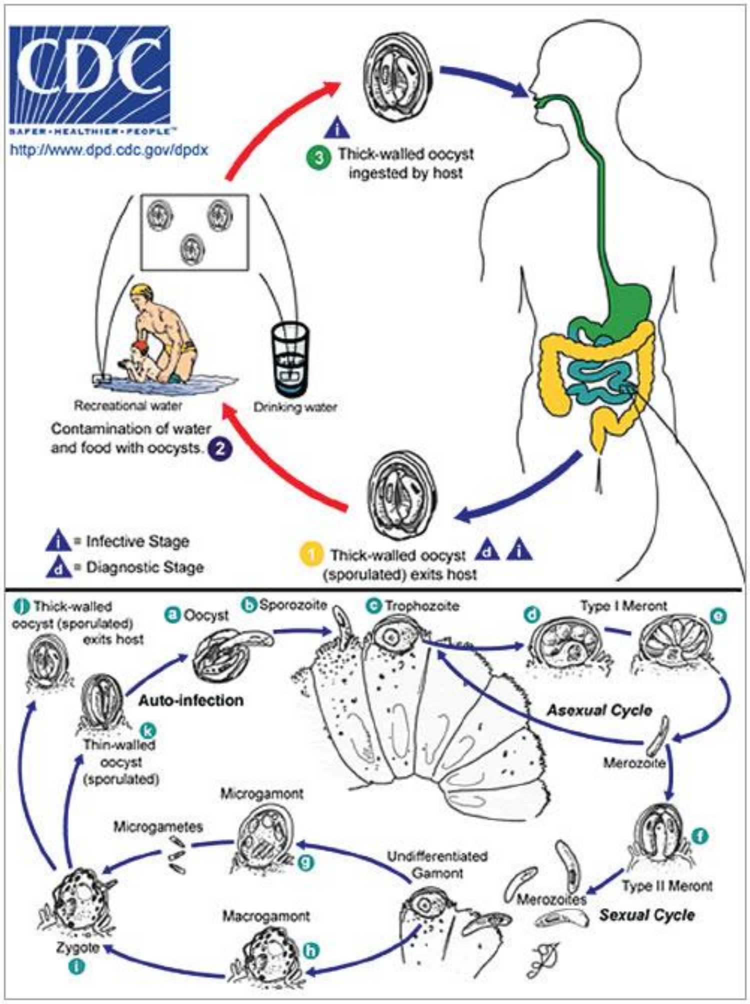

Figure 1: Life Cycle of *Cryptosporidium parvum* and *C. hominis*

WHAT YOU CAN DO

Water conditions designed to meet human consumption standards may not necessarily be adequate for research models. Certain contaminants could impact the research being conducted, adversely affect animal health, and ultimately require more vet and employee time. A facility could even be faced with the cost of replacing study animals. Where possible, stay informed. Figure 2 is a water quality comparison that illustrates the possibilities for gaining information.

Water Quality Analysis Comparison
City of London *vs* English Research Facility

Test Parameters	Footnote	Units	City of London Drinking Water: Average	City of London Drinking Water: Max	vs English Research Facility (Pre-Treatment)
Colour (Pt/Co scale)		mg/lpt/CO	2.4	4.7	0.4
Turbidity (clarity of water)	1	FTU	0.12	1.25	0.05
Conductivity at 20C	2	µS/cm	604	652	694
Ammonium as NH_4	3	mg/l	0.12	0.17	0.009
Nitrate as NO_3	3	mg/l	27.1	36.5	34.4
Nitrite as NO_2	3	mg/l	0.026	0.11	0.003
Odour (quantitative)		Dilution no.	0	0	0
Taste (quantitative)		Dilution no.	0	0	0
Bromoxynil	4	µg/l	0.002	0.003	0.007
2,4-D	4	µg/l	0.002	0.002	0.003
Dicamba	4	µg/l	0.004	0.004	0.006
Dichlorprop	4	µg/l	0.003	0.003	0.003
Ioxynil	4	µg/l	0.001	0.001	0.004
Mecoprop	4	µg/l	0.003	0.007	0.003
MCPA	4	µg/l	0.002	0.004	0.003
MCPB	4	µg/l	0.002	0.004	0.004
2,3,6-TBA	4	µg/l	0.004	0.004	0.006
2,4,5-T	4	µg/l	0.002	0.002	0.003
2,4-DB	4	µg/l	0.004	0.004	0.005
Total Pesticides	4	µg/l	0.033	0.131	0.011
Tetrachloroethene	5	µg/l	0.1	0.2	0.02
Trichloroethene	5	µg/l	0.1	0.2	0.08
Tetrachloromethane	5	µg/l	0.1	0.2	0.02

Below London's average and high level (no action needed)
Below London's high level but higher than average level (further monitoring needed)
Above London's high level (concern, may have an impact on the research)

London is subject to Water Quality Regulations for England and the European Union.

[1] Clarity of water is affected by silt or biologicals in the water.

[2] Conductivity is how much electricity can pass through the water; the higher the number, the dirtier the water. Treatment would be recommended in this case.

[3] The source of Nitrates are fertilizers found in run off water. Typically, during the Summer the Nitrates are higher when fertilizers are being used.

[4] Pesticides are found in run off water. Each pesticide could have a different impact on the animals and the research being conducted.

[5] A possible source of Organic Chemical is disinfectant by-products.

This is a partial list of test parameters for a water quality analysis.

Figure 2: Water Quality Analysis Comparison: City of London vs. English Research Facility

Analyse

You see how easily specific studies using certain animal models could be skewed by what is in the water if the water is not filtered, purified, and treated properly. Notice conductivity and total pesticides. We monitor conductivity— ease of electricity transfer— because the conductivity increases with contamination. The level reported in this water test is higher than the average reported by the City of London in their annual water test report. The fact that pesticides were detected means the water was contaminated at some point by runoff. The ability to remove and/or reduce variables from the research is paramount. The effect that the pesticides can have on the research will vary depending on the research being conducted and the animal models being used.

When analysing your current water supply, one of the best and easiest places to start is with your local water district. Most water districts publish annual reports on their websites. These annual reports give details about the quality of the water they deliver. Though these numbers are for the system at large and not specific to your facility, it is still a good place to start the water analysis process.

When you conduct analyses, you begin to set standards for your facility that meet your research needs. As a result, your water conditions become consistent and repeatable.

You’re actually providing consistency throughout your entire facility—ensuring that all rooms, racks, and cages receive equal quality water.

Having water quality data enables researchers to reproduce exact water conditions in other facilities. Therefore, research can be repeated or duplicated in the same facility or anywhere in the world. Providing consistent and repeatable water quality makes the researcher confident that water remained the same and within specification for the duration of their study.

Test Regularly

A good guideline is to test the water twice per year. January and August are ideal times. If the municipal water source is from open water and is in an area that can have freezing temperatures, water sources will freeze, and the municipality may switch to a well or some other water source. In the summer, they may switch back to their original water source, and the facility may start receiving runoff from rain and other sources, therefore the water quality continues to change (Figure 3).

Why Test?

It's important for a facility to test water because the standards set by the water quality regulations around the globe are designed to provide what is acceptable for human consumption, not for the research animal. That is why each facility should create their own water quality standard based on the research being conducted.

When to Test?

Water varies seasonally and even daily. Even though a facility tested their water at the beginning of the year it may not be identical to what it's receiving now.

What to Test?

A facility should plan to test twice a year, January and August being the best times. This is based upon a municipality's seasonal water source changes.

Most water testing labs have a standard list of items that they test for including elements and minerals, viruses and bacteria, as well as the present condition of the water. A facility can customise their report as needed for their research needs.

Where to gather water samples?

A facility should pull samples from three key areas. The incoming water to the facility before treatment, immediately following treatment, and at the farthest point in the vivarium from treatment.

The testing lab that a facility contracts with will be able to provide a kit. Samples need to be gathered and sent back to the testing lab within 24 hours.

Figure 3: Guidelines for Water Quality Testing

Purify

It is essential to purify and treat the water that research animals will drink, and there are several methods from which you may choose. It is not within the purview of this piece to set out the benefits of each or the specific circumstances within which one or the other is preferable. However, for purposes of comprehensiveness, the key purification methods are:

Filtration: Filters of decreasing porosity are placed in series to remove particulates and other large contaminants. Carbon filtration is used to remove organics and chlorine.

Ultrafiltration: Ultrafiltration provides a level of purification between particle filtration and reverse osmosis, eliminating viruses and bacteria while not removing most minerals.

Reverse Osmosis: Reverse osmosis (RO) provides fine filtration with a result of >99.9% of most contaminants being removed including viruses, aqueous salts, pyrogen, and metal ions. Reverse osmosis is a process of forcing water through a microscopic filter/membrane (<0.001 microns) using high pressure. As the water passes through the membrane the contaminants are removed. The purified water is then stored with a residual disinfectant such as acid or chlorine and is ready to be delivered to the animals via an automated watering system (Figure 4).

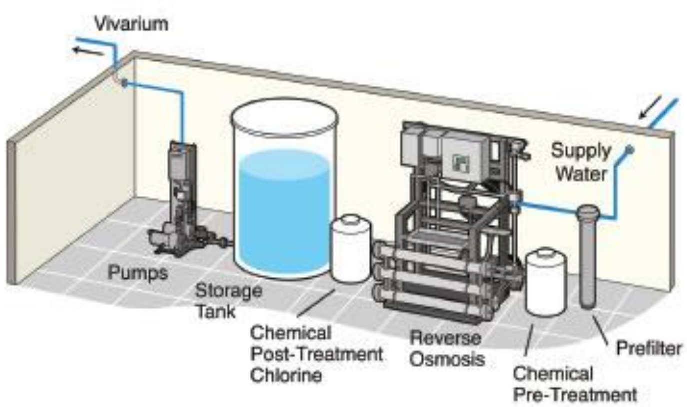

Figure 4: Water Purification and Treatment Process

STANDARDS FOR REPEATABLE RESEARCH DATA

In an attempt to eliminate or minimise the number of potential variables in the drinking water supplied to laboratory animal models, regardless of the study design, the drinking water provided by each animal facility should conform to a standard. This standard should be applied to the drinking water offered to every animal model involved in a research project, thereby protecting the integrity of the study and obviating the extrapolation of the data that is generated based on a varying water quality.

In the event the research community does not establish a strict quality standard for animal drinking water, the ability to generate repeatable research data could be compromised. I feel it is imperative that the animal care industry develop more stringent standards for drinking water quality. We already define the nutritional content for the food given to the animals; it is time that we define the content of the other source of nutrients and minerals: water.

Bob Lemken *is Business Development Manager for Edstrom Industries in Waterford, Wisconsin, USA. He has been a member of the American Association of Laboratory Animal Science (AALAS) and its predecessor, the Animal Care Panel. Bob has a degree in Animal Science from the University of Maryland. His involvement in laboratory animal care began in 1963 at Johns Hopkins University in Baltimore, Maryland, USA.*