

**Gesellschaft für  
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**GV SOLAS**

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## **Working Group on Nutrition**

# **Drinking water supply for laboratory animals**

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## 1 Introduction

Water is essential to life. Acute water losses of approximately 5 % of body mass can lead to a definite reduction in performance; over 10 % can cause serious to life-threatening metabolic dysregulations. For this reason, laboratory animals must be supplied with water 24 hours a day in a manner that is appropriate to their species. The dry feed regimes that are now standard require drinking equipment that allows every animal to consume a sufficient amount of liquid at all times.

Depending on the animal species and the type of management, different requirements must be set for the quality of the water as well as the dispensing method.

## 2 Quality of the water

### 2.1 Drinking water quality (according to the "Drinking Water Ordinance")

Laboratory animals that are kept under conventional conditions can generally be given drinking water from approved water supply systems. Exceptions to this are investigations in which no ingredients may be provided with the water (e.g. mineral balances).

The Ordinance on the Quality of Water for Human Consumption (TrinkwV) 2001 – Drinking Water Ordinance – of 21.05.2001 (Federal Law Gazette BGBl. I No. 24 of 28.05.2001 p.959) regulates the requirements, guide and limit values as well as permissible deviations in Germany. The hygienic data to be monitored includes:

- **microbiological parameters:** a maximum of 100 germs may be detected in 1 ml of water, and no Escherichia coli, no coliform bacteria and no enterococci may be contained in 100 ml of water, according to a limit value definition, (cf. Appendix 1 TrinkwV 2001, General Requirements for Water for Human Consumption)

and

- **chemical parameters:** the permissible limit values of chemical parameters in the distribution network of a house installation are listed in Appendix 2 of the TrinkwV.

The monitoring is done by local public health departments or authorities commissioned by them.

Testing is done by approved laboratories that have been accredited for this purpose.

## Notes:

- The ordinance allows deviations from this standard when e.g. geographical particularities cause the water quality to change and the deviations are not dangerous to human health. In emergency cases, temporary deviations are possible. For this reason, it is always advisable to obtain information on the water quality before beginning experiments.
- The quality of pipes in a building must be taken into consideration. Pipes can greatly affect the water quality, often in a negative way. With old pipework, there is a risk that heavy metals will leach in. It can therefore cause problems when water lies in the pipes for a period of time. Before water is used from these pipes, it should be run out (estimate the length of time based on the length of the pipes). If drinking water is treated to improve its hygienic status, the condition and the age of the pipes must also be taken into account, depending on the method used.
- In literature, there are indications that the water quality can show differences that are related to the location (Guide for the Care and Use of Laboratory Animals, 1996; Newell, 1980). For example, more minerals are to be expected in drinking water from ground water than in drinking water obtained from surface waters (rivers, lakes, dams). Furthermore, metal pipes are a potential source of minerals as well as of organic chemicals (Newell, 1980).
- Different authors give different recommendations for regularly testing drinking water, depending on the water quality to be achieved. These range from checking the water and water surfaces for coliform bacteria and *Pseudomonas aeruginosa* every week (Thibert, 1980) to checking the water supply points once or twice a year according to the criteria of the TrinkwV.

Before an experiment begins, the person responsible for animal management should have the local water quality documented by the local water supply companies (waterworks) or test the water at the taps.

## **2.2 Demineralized water/partially desalinated water ("soft water")**

This water is not necessarily low in germs. In this case, only the mineral content of the water is changed, which may actually encourage rather than hinder the subsequent growth of germs. The germ level depends on the hygienic condition of the treatment system and the standing time of the water before it is used as drinking water. Further hygienic treatment (see points 2.3 and 2.4) may be necessary, depending on the application.

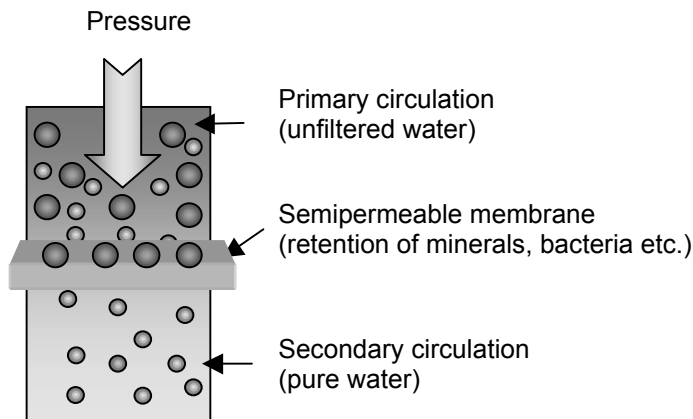
On the other hand, the demineralization/partial desalination of water can also be an important pretreatment for subsequent water processing, especially in the case of very chalky (so-called hard) water. In such a case, it is a good idea to demineralize water before it is autoclaved or used in automatic drinking systems, in order to prevent the drinking nipple from becoming blocked.

Since demineralized water has a strong "urge" to absorb minerals, it has a very aggressive effect on metals and alloys. The material that is selected for pipes and fixtures must meet the regulations for demineralized water. Partially desalinated water is less aggressive, depending on the degree of desalination.

### **2.2.1 Reverse osmosis**

Reverse osmosis is a method for demineralizing water. Sterile drinking water cannot be produced with reverse osmosis methods, however. Even when a system functions optimally, a small amount of germs will always remain. It is recommended to consistently separate the primary and secondary circulation, because there will otherwise be a risk of contaminated water from the primary circulation entering the clean secondary circulation. If the basic technological information and maintenance of the system are not observed, the sealing rings can become a weak point in the reverse osmosis system. Problems then occur with germ contamination in downstream pipe systems, either in a retrograde way or through "seepage water" from the primary circulation. Usually, colonization with pseudomonads occurs first. These form a biofilm that is in turn a good breeding ground for pathogenic germs. The main application field for reverse osmosis is the area of dialysis. It is definitely inadvisable to use reverse osmosis as the sole method for the hygienic treatment of drinking water in SPF facilities. Reverse osmosis should be combined with other water treatment methods, e.g. with an ion exchange system and a method for producing low-germ or germ-free water (p. 2.3, 2.4). This results in costs for several treatment systems (Brandstetter, 1998a).

*Conclusion: Reverse osmosis is technically complicated and can be used to demineralize water, but it does not produce sterile drinking water. Therefore, this method should only be used in combination with other methods, e.g. heating or acidification.*



**Fig. 1 Schematic diagram of the principle of reverse osmosis**

### 2.2.2 Ion exchange

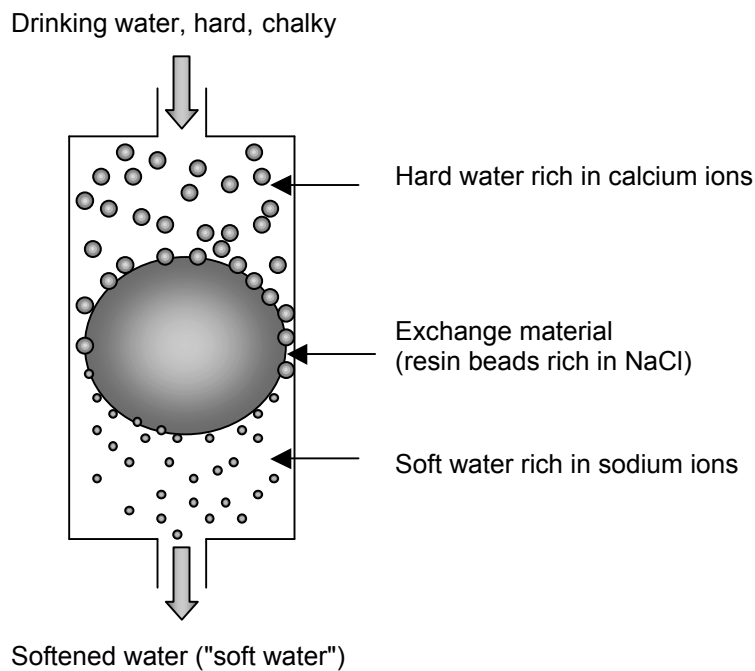
Various minerals are removed from the drinking water using an ion exchanger. Depending on the ion exchanger resins that are used, different water qualities can be produced, from partially desalinated water ("soft water") to completely desalinated water (demineralized water).

Cation exchangers are often used for softening. These extract  $\text{Ca}^+$  and  $\text{Mg}^+$  ions from the drinking water. The method is based on an exchange of  $\text{Ca}^+$  and  $\text{Mg}^+$  ions with  $\text{Na}^+$  ions. So-called "soft water" results with a higher share of bonded  $\text{Na}^+$  ions than in drinking water.

Complete desalination (demineralization) is based on the same active principle.

Water is desalinated using ion-exchanger resins that are embedded between ion-selective membranes. These membranes function as anion-permeable or cation-permeable membranes. The membranes function as a barrier against the main water flow but allow the ions to pass. That means that the cation-permeable membrane (usually  $\text{H}^+$  as a reactive group) can be passed by cations, but not by anions. Correspondingly, the anion-permeable membrane (usually  $\text{OH}^-$  as a reactive group) can be passed by anions, but not by cations. This process can take place under the influence of an electrical field (e.g. also in the case of water treatment in special installations for laboratory needs) or when pressure is applied. The drinking water that is fed in continuously emits ions, which are collected in the exchanger resins or in concentrate channels. Their capacity determines the effectiveness of the system. The exchange results in completely desalinated water (Millipore, ELIX, Wasseraufbereitungssysteme, 1996).

*Conclusion: The production of partially or completely desalinated water for drinking purposes involves additional costs. Such water qualities are often present in animal management operations, since they are also needed for other equipment (e.g. cage-washing equipment, autoclaves). In this case, only a small amount of extra costs would result for the drinking water treatment. The use of demineralized/partially desalinated water for drinking purposes basically depends on the quality ("hardness") of the drinking water and the other methods for hygienic treatment (e.g. heating) and the type of water supply (e.g. automatic drinkers). This method does not necessarily produce sterile water.*



**Fig. 2 Schematic diagram of the principle of water softening on the basis of ion exchange**

### **2.2.3 Distilled water**

The use of distilled water is common above all where autoclaved water is used for drinking water. It provides the technical advantage that storage vessels and water bottles do not "clog up", especially in the case of very hard tap water. However, this is not necessarily water that is low in germs. With this type of water treatment as well, the germ level depends on the cleanliness of the treatment system and the time that the water stands in storage containers until it is used as drinking water. A further hygienic treatment depending on the application is unavoidable. When used in central drinker systems, the regulations for pipes and fittings must be observed in the same way as for demineralized water. Even in the case of lifelong administering of distilled water, no negative effects or deficiencies have been observed in the animals or their descendents. The mineral requirements are apparently sufficiently met by the feed, just as with tap water that is very low in minerals. The use of distilled water involves increased costs and is only worthwhile in special applications, e.g. when animals are kept in isolators. Distilled water and water that has been demineralized using ion exchangers are of equal value as drinking water for laboratory animals (Lorenz, 2001).

*Conclusion: Distilled water is generally suitable as drinking water. It is equal to water that has been demineralized with ion exchangers. It is costly and can therefore only be recommended for special applications (see also 2.4.1 Heat sterilization). This method does not necessarily produce sterile water.*

### **2.3 Water low in germs**

Various water treatment methods can be used for investigations in which the contamination of water with germs (acc. to 2.1) could be a source of interference, but the use of sterilized water, on the other hand, would be too costly and is not necessary.

#### **2.3.1 Chlorination**

The chlorination of drinking water is a simple, inexpensive and proven method. It is by far the most commonly used one. In this case, chlorine gas or other chlorine compounds (such as sodium hypochlorite or chlorinated starch with a high degree of purity) are added to the water via dosing pump systems, which causes a reaction with organic substances (chlorine can also be added manually). The remainder or surplus of chlorine ions leads to the disinfection or even sterilization of the water.

Chlorine works fast to kill germs and has a sustained effect in the pipework due to its gradual binding to particles in the water.

Chlorination that is too strong can lead to offensive odors and irritated mucous membranes in humans and animals (Thunert et al., 1975; GV-SOLAS publ. no. 5/6, 1988).

In commercial breeding facilities, the chlorination method is often used to treat drinking water (usually in combination with other methods, e.g. acidification). Chlorination can be done with sodium hypochlorite. Experience teaches that the active chlorine becomes effective at a pH value of 5.0. The concentration of free chlorine should be 6-8 ppm; in combination with acidification to the pH value of 5.0, water can be obtained that meets the requirements for SPF management conditions (Leblanc, 2002).

Occasionally, 15-20 ppm of chlorine are recommended for mice management, although Bywater et al. (1977) were able to keep water bottles free from bacteria for 7 days with only 5 ppm of chlorine in the drinking water. Homberger et al. (1993) achieved freedom from *Pseudomonas aeruginosa* in mice (fecal evidence) with 6-8 ppm of active chlorine. The water bottles were changed once a week. If the bottles are changed more frequently or an automatic drinker system is used in connection with an automatic chlorine gas system, the chlorine concentration could be even lower. At the time the bottle is changed, the chlorine concentration should never be below 2 ppm.

*Conclusion: The chlorination of drinking water is a very effective, proven and inexpensive method. It should be used with a reasonable expense. The combination with other methods to increase the germicidal effect should be reconsidered (acidification, filtration).*

### 2.3.2 Acidification

Another possibility for water treatment is acidification, which produces drinking water that is low in germs. Sterility is not achieved, however! Almost all types of germs grow more slowly at a pH value of 2.0 to 3.0. A measurable increase does not occur until late (after approximately 3 to 5 days). The success in preventing the spread of above all gram-negative types of germs (in particular *Pseudomonas aeruginosa*) is good, although these germs are not killed either.

Different acids can be added to the drinking water (sulfuric acid or hydrochloric acid, Hall et al., 1980; peracetic acid, Juhr et al., 1978); acidification with hydrochloric acid is the most common form. There are varying opinions on the influence of acidification on laboratory animals. Hall et al. (1980) found an effect on the germ level in the small intestine, depending on the acid used. Acidification to a pH value of 2.0 with sulfuric acid reduced the number of bacteria isolated from the terminal ileum more than with acidification with hydrochloric acid. However, mice that received acidified water generally had a significantly lower weight development after 6 weeks of treatment in comparison to mice with normal drinking water.

Karle et al. (1980) found that acidification of drinking water with hydrochloric acid to a pH value of 2.0 to 3.0 attacks the tooth enamel of the laboratory animals, although the mixed saliva of rats normally shows a pH value of 8.0 – 9.0 and has a high buffer capacity.

Clausing et al. (1989) recommend not lowering the pH value under 3.0 (rat); at a pH value of 2.0, they were able to observe a slight proteinuria with a reduced volume of urine.

Other authors were not able to determine any health impairment after acidified water was administered (Tober-Meyer et al., 1981; rats, rabbits).

A further problem that must be taken into account with this water treatment method is the selection of usable materials. A disadvantage is that a large number of materials are not resistant to the acids and will corrode, for example drinking nipples and cages made of galvanized sheet metal. For this reason, stainless steel or plastic material must be used. Copper, brass and most aluminum alloys cannot be used, since they corrode (GV-SOLAS publ. no. 5/6, 1988).

If bottles with stoppers are used, the leaching of minerals from the stoppers can be greater with deionized and acidified water than with untreated water. Kennedy et al. (1991) recommend plastic stoppers made of red vinyl or silicone, which are more expensive than the usual black stoppers. The mineral discharge from new stoppers is distinctly higher than from used ones that have been washed several times.

Combinations of acidification and other methods produce a good water disinfection. In this way, the water can first be acidified with hydrochloric acid to a pH value of 5.0 via dosing pumps; later, chlorine can be added via dosing pumps as well (6-8 ppm active chlorine). A (usually automatic) readjustment of the pH value is necessary. There are companies that offer technology for this.

*Conclusion: Acidification is a suitable method for producing water with a reduced amount of germs. It requires the use of corrosion-resistant materials and therefore has high resulting costs. In combination with other methods (such as chlorination), water can be sterilized.*

### **2.3.3 UV radiation**

UV disinfection is approved for disinfecting drinking water. With this method, UV radiation emitters produce the disinfecting ultraviolet light in flowing water. The required steady radiation can be attained with a turbulator. UV light in the wavelength range from 200-280 nm has a germicidal effect, whereby the maximum effectiveness is at 260 nm. The nucleic acids of the genetic material of the microorganisms also absorb the maximum amount of light at this wavelength, so that their DNA and RNA are changed by UV light. This causes the microorganisms to lose their ability to reproduce. UV disinfection does not require long dwell times, since these processes take place in fractions of a second. The radiation dose should be at least 40 mJ/cm<sub>2</sub> at the end of the service life of the radiation emitter; this reduces germs by 10<sup>5</sup>. The UV light generated by the UV radiation emitter should not be below 240 nm, in order to prevent the generation of ozone and the formation of harmful by-products in the water to be disinfected (BWT info, 1998).

UV disinfection systems are compactly structured, stainless-steel flow reactors that are easy to handle. Equipment should be rinsed during water stagnation (automatically if possible). UV disinfection systems are used, for example, to disinfect drinking water for municipal and private areas as well as in the beverage, food and pharmaceutical industry (BWT info, 1998).

Publications from the 1970's and '80's assume that drinking water cannot be successfully sterilized with UV radiation (Thunert et al., 1975; GV-SOLAS publ. no. 5/6, 1988). The problems are seen in the following: the germicidal effect of radiation especially depends on the composition of the water and the length and intensity of the radiation.

If many organic materials and mineral salts are present in the water, the radiation will only penetrate slightly. Germs that are embedded in dirt particles or that occur in colonies will not be reached by the radiation.

*Conclusion: This method is advisable as an environmentally-friendly way of disinfecting drinking water without changing the water in any way. Whether sterility is achieved depends on the system and the current conditions (pipework, drinking water quality etc.). For SPF systems, UV radiation of the drinking water cannot provide sufficient safety as the sole method.*

### **2.3.4 Ozone treatment**

Ozone is a particularly energy-rich form of oxygen and is produced directly before it is fed into the water. Ozone (O<sub>3</sub>) contains one oxygen atom more than (standard) oxygen (O<sub>2</sub>). Ozone is used as a disinfectant and an oxidant; it acts as a bactericide, virucide and fungicide. Ozone also oxidizes registered organic and inorganic contaminations without forming troublesome reaction products. Pure oxygen (O<sub>2</sub>) is formed as a residue (BWT info, 1998).

Ozone treatment requires a special system that usually involves high acquisition costs. The principle of the systems is based on generating ozone from air using electrical current. High-quality materials must be used, such as housings made of stainless steel and special glass (breakage safety). The supplied air must be dried and, if necessary, cleaned with absorbers (or by using highly effective, macroporous molecular sieves). The regeneration of the driers influences the service life of the ozone generators. The heat that is produced during ozone generation is cooled down; the indirect cooling prevents water from entering the high-voltage area even in the case of glass breakage.

The amount of ozone that is produced can be adjusted as needed at any time. The ozone is produced and transported at a negative pressure (vacuum) for safety reasons. The ozone must be intensively mixed with the treated water in special ozone/water mixing systems (to ensure ozone solubility and avoid exhaust gas losses, BWT info, 1998). The ozone can also be added to the water via so-called inoculation points (Bieniek et al., 1981). A measure for the germicide rate as well as for the viability of germs in water is the redox value; the germicidal effect increases along with the redox potential. In the case of Bieniek et al. (1981), the redox values varied between 580 – 780 to maintain bacteriologically germ-free water. According to his experience, water containing ozone can disinfect pipe systems up to the drinker nipples. Despite these possibilities, Bienek et al. (1981) recommend the combination with a further water treatment method (e.g. acidification).

Residual ozone must be eliminated from the water, either with residual ozone converters (activated carbon or catalysts) or through UV radiation as well (Thunert et al., 1975; Bieniek, et al., 1981; BWT info, 1998).

*Conclusion: Due to the high costs, this is not advisable for small animal facilities. Use in combination with other methods is recommended.*

## **2.4 Germ-free water**

Gnotobiotic and germ-free animals must only be supplied with germ-free water. The use of germ-free water is also recommended for specified pathogen-free animals. Water sterilization methods that produce a provable sterility of the water must be used.

### **2.4.1 Heat sterilization**

The following points must be observed for sterilizing drinking water in autoclaves:

- Drinking water can be sterilized in the water bottles.
- Depending on the type of drinker caps that are used (size of the opening), the caps can pop off or the water bottles can be damaged as a result of the prevailing pressure conditions during sterilization when bottles are autoclaved with the caps on. It is therefore recommended to autoclave water in bottles without their drinker caps and not to put the caps (which have also been sterilized) back on until after sterilization.
- Some of the mineral salts that have been dissolved in the drinking water are eliminated during heating (carbonates). The eliminated "chalk flocks" can block the openings in drinker caps. The drinking water should therefore be softened before sterilization; demineralized or distilled water is also well suited. Very soft water is naturally an exception.
- The material of the water bottles that are used can influence the sterilization temperature. Makrolon bottles can be autoclaved up to a maximum of 120-125°C, according to the manufacturer's information.  
 Since the attainment of sterility depends on the pressure, time and temperature, it is possible to obtain sterile material even at temperatures under 121°C by varying the parameters. Experience shows that the wear of Makrolon can be considerably reduced at lower temperatures. Makrolon bottles are therefore often autoclaved at temperatures of only 116-118°C but for longer periods. Good experiences have been obtained with the sterilization of drinking water in Makrolon bottles at 116°C and a period of 40 minutes as well as at 118°C and a period of 20 minutes. This data is based on a particular equipment type (MMM Vivilab 14L14) and should be checked for all other devices (see also GV-SOLAS, Committee for the Nutrition of Laboratory Animals, yellow booklet "Hygienische Behandlungsverfahren für Futtermittel, Dampfsterilisation von Futter im Autoklaven" [Hygienic treatment methods for feedstuffs, steam sterilization of feed in autoclaves], 1998).
- When drinking water is sterilized in bottles, the relatively long heating time and above all the long cooling process after sterilization must be taken into account, depending on the total amount of liquid in the autoclave. Large quantities of water (200 liters and more) are therefore best autoclaved overnight. The total duration of the program naturally also depends on the equipment specifications of the respective steam sterilizer itself and the available steam and cooling capacities. Before the sterilized material is removed from the steam sterilizer, it is imperative to ensure that the system has cooled down sufficiently. The interior temperature of the autoclave must be below 80°C before the system can be opened; otherwise a defervescence can occur (Brandstetter, 1998b).

*Conclusion: This type of water treatment is advisable when animals are kept in isolators, since this generally requires an autoclave. Due to the long time period of the sterilization process, this method can only be used for limited amounts of fluid and is unsuitable for automatic drinker systems.*

### **2.4.2 Sterile filtration**

Minimum-pore filters were used for the filtration devices. A filter pore diameter of 0.2 µm is needed to achieve sterility (Thunert et al., 1983; Brandstetter, 1998c).

A prefiltering system is recommended to prevent the filters from being quickly contaminated.

Depending on the water quality, several stages are connected in series. This allows most particles (inorganic and organic, esp. bacteria) up to a size of 2 µm to be separated without stressing the sterile filter (e.g. Pall or Hosch filter). In addition, it is also possible to use water that has been softened or completely desalinated with ion exchangers (see 2.2.2).

Newer filter systems can be directly mounted at the water supply point and prevent germs from growing through the filter membrane with special technology (Warncke, 1998).

The filters are only effective if they are maintained carefully and correctly. If this is not done, they are more likely to inflict damage, since they then become a germ reservoir (observe the manufacturer's instructions).

The prerequisite for successfully using filter devices is a reliable sterilization of the filters and the pipework behind the filter. This should be kept as short as possible. If used continuously, the filters should be sterilized at least twice a week. If water is not withdrawn continuously, filters should be sterilized before each use (in an autoclave). Depending on the type of filter, they can be autoclaved between 3 and 30 times; afterwards, the function of the filter must be tested each time (special tests are available; carry out filter sterilization and tests according to the manufacturer's instructions). Filters are not suitable for discontinuous use without constant checking.

In addition to checking the filters, it is recommended to test purity of the drinking water at regular intervals (every week to every 14 days) (Raynor et al., 1982).

Due to the fine-pore filters, water must pass through the filter system with a corresponding pressure. All filters cause an increasing pressure loss in the pipe system (Thunert, 1975). To achieve a sufficiently high water pressure (depending on the existing pressure in the drinking water network), an additional pressure-increasing installation may be necessary (Brandstetter, 1998c).

*Conclusion: Sterile filtration is only practical and effective when the filters are at the end of the pipework. Such a system requires a great deal of maintenance, but is reliable and economical with regular maintenance.*

### **2.4.3 Combined demineralization/decontamination methods**

The combination of water softening (ion exchanger/reverser osmosis), subsequent sterilization by heating to 134°C, followed by acidification with HCl to the pH value 2.7 is an efficient method for large systems. However, this type of water treatment requires a great technical effort (tanks and dosing

systems), avoiding excessive standing times in the pipes (e.g. automatic flushing at night) as well as regular checking of the parameters.

Without a desalination effect, the combination of heating the drinking water (to approx. 80 - 95°C) and the subsequent acidification (pH value 2.5 – 3.0) has proven while maintaining the SPF status over several years (Thunert et al., 1975).

*Conclusion: In the case of large animal facilities, the combination of methods should be selected depending on the available resources (technical and financial) as well as depending on the required water quality.*

### **3 Method of water dispensing**

Water is provided either through individually used drinking vessels or via automatic drinking systems. In addition to the technical adaptation of the vessels to the management system, the only condition is that the animals must have free access to the drinking equipment. If the experiment requires the water consumption to be registered, graded drinking vessels must be used.

#### **3.1 Drinking vessels**

Buckets and bowls (for larger laboratory animals such as sheep, goats, pigs, dogs and cats) or bottles can be used as drinking vessels that often have to be filled individually by hand. Water bottles are suitable for almost all laboratory animals (up to 3 kg body weight). The drinking nipples have different designs.

Water bottles with short and/or long nipples are used for rats, guinea pigs and mice. Long nipples have the advantage that recently weaned animals can also reach the water. Short nipples are more suitable for larger animals (rats), since animals do not accidentally touch them so often (causing water loss). The filling level of the water bottles must be checked regularly. This provides the experimenter with information on the drinking behavior (well-being of the animals) and on the functioning of the water bottles. If they are not closed properly or when pressure variations exist in the animal room, it is possible for the water to run out. Young, wet mice can get hypothermia very fast, become sick and die. Animals can also die of thirst, though, with a full water bottle. New drinking nipples can have sharp edges that prevent the animals from drinking, or the water can be prevented from flowing by nipples that have not been degreased correctly. Litter material and food residue can block the water opening. If there is no air bubble in a full bottle, small mice will have difficulty drinking water from the bottle. It is recommended to change and clean bottles and drinker caps at least once a week (GV-SOLAS publ. no. 5/6, 1988).

#### **3.2 Automatic drinking systems**

Automatic self-drinkers can be used for all laboratory animal species. Bowl or nipple drinkers are practical, depending on the species.

The functioning of drinker nipples must be checked manually on a regular basis. Drinker nipples that continuously drip can fill up plastic cages with water overnight and cause the animals to drown. It is hardly possible to clean the nipples of an automatic water supply system while it is operating. In this case, it is necessary to wait until a general cleaning of the room or the shelf (GV-SOLAS publ. no. 5/6, 1988).

### **3.3 Moist feed**

The administration of moist feed has proven itself in special situations. For example, water consumption can be made easier for recently weaned mice by additionally supplying moist feed (soaked pellets), e.g. in a Petri dish or directly in the cage litter. It must be taken into account that mold can develop if uneaten feed is not removed in time. It is also recommended to add moist material when animals are being transported for several hours. The simplest method is to soak laboratory animal feed (low-germ/germ-free pellets) in a vessel overnight and then put it in the transport container. Some breeders use moist feed that is sterilized in plastic casings (sausage-like shape) or gelatin that is shrink-wrapped in plastic, which is also commercially sold as "solid drink". This plastic casing has to be torn open before use, in order to allow animals access to the feed.

It is not advisable to use raw potatoes, carrots or apples for SPF animals. An alternative is to supply potatoes that have been sterilized in preserve glasses (Hagelschuer, 1998).

Home-made gelatin (water + gelatin, let soak, heat to 70°C and then let cool down) is also well suited to be given to animals during long transports (Thomae, 2001).

## **4 Recommendations of the committee**

For the care of conventional animals, the quality of drinking water from water supply systems in accordance with the Drinking Water Ordinance is usually sufficient. For the care of SPF animals in a breeding operation or for particular experiments (e.g. measurements of the mineral input), however, it is necessary to treat drinking water for demineralization and/or germ reduction/sterilization.

For gnotobiotic and germ-free animals, the water must be sterile.

With all methods of water treatment, it is necessary to ensure that drinking vessels are cleaned or replaced regularly, since they will otherwise become contaminated with germs from the oral flora of the animals that come into contact with the drinking vessel!

Depending on the available technology (e.g. pipes) and financial resources, the following methods are recommended:

To achieve a low germ level in the water:

1. Chlorination (most economical method)
2. Acidification to pH value 2.5 – 3.0 (expensive because only certain materials are acid-proof)
3. Combination of several methods (often very complicated)

To achieve sterilization of the water:

1. Heat treatment (most reliable method, but requires a steam sterilizer)
2. Filtration

To demineralize water:

Ion exchange

Before setting up one's own water supply, it is advisable to look at water treatment systems in other lab animal facilities, in order to obtain manufacturer's information and practical tips. The water treatment should be adapted to one's individual needs together with the manufacturer, due to their knowledge of the methods.

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