GV-SOLAS
Gesellschaft für Versuchstierkunde
Society for Laboratory Animal Science

Specialist information

from the Committee for Nutrition of Laboratory Animals – (GV-SOLAS)

Provision of Drinking Water for Laboratory Animals

Status March 2016

Authors: Ina Hagelschuer,
Heike Wagner, Günther R. Warncke

Content

1. Introduction

Water is vital for life. Acute losses of water amounting to about 5% body mass can lead to a marked decline in fitness, from more than 10% to serious and life-threatening metabolic dysregulation. For this reason, a species-appropriate supply of water for laboratory animals must be guaranteed 24 hours a day. Today's standardized dry feed regimen calls for watering facilities that offer each animal the possibility of an adequate liquid intake at any time of the day. Requirements differ with regard to water quality and to the manner in which it is supplied depending on the species and the form of housing.

2. Water quality

2.1. Drinking water quality (according to "Drinking water ordinance")

Laboratory animals housed under conventional conditions can usually be provided with drinking water from approved water supply systems. Exceptions are in the case of studies where no ingredients must be supplied with the water (e.g. for mineral balance).

The quality for water for human consumption is in Germany regulated by law, e.g. with threshold limits for microbiological and chemical parameters (Drinking water ordinance and analogous legal provisions in their current versions; see also Annex 1).

Monitoring is conducted by the health authorities or offices commissioned by these authorities. Investigations are conducted by accredited laboratories approved for these investigations.

Notes:

- The ordinance allows for deviations from the norm if e.g. geographical peculiarities cause a change in the water quality and the deviations are not hazardous to human health. In emergencies, time-limited deviations are possible. It is therefore always advisable to obtain information about water quality before the start of an experiment.
- The quality of piping in the building must be borne in mind. Piping can have a major - often negative - impact on water quality. With old piping systems, there is a risk of heavy metals entering the water. The length of time during which the water is left standing in the pipes can therefore pose problems. Before water from these pipes is used, it is recommended to let the water run for some time (estimate the time needed according to the length of the pipe). If the drinking water is treated to increase its hygienic status, the condition and age of the pipes must likewise be borne in mind depending on the treatment process used. Metal piping is also a potential entry source not only for minerals, but also for organic chemicals (Newell, 1980).
- In the literature, there are indications that water quality can differ depending on the location (Guide for the Care and Use of Laboratory Animals, 1996; Newell, 1980). For example, more minerals can be expected in drinking water from the groundwater than in drinking water obtained from surface water (rivers, lakes or reservoirs).
- Different authors offer differing recommendations for the regular inspection of drinking water depending on the water quality to be achieved. These range from a weekly check on water and water bottles for coliform bacteria and Pseudomonas aeruginosa

(Thibert, 1980) to an inspection of the water outlet points once or twice a year according to the criteria of the Drinking Water Ordinance (TrinkwV).

- Before the start of an experiment, the person responsible for animal housing should get the customary water quality documented by the local water supply companies (water utilities) and test the water at the taps.

2.2. Demineralized water/partially desalinated water ("soft water")

This water does not necessarily have a low microbial count. With this kind of water, the mineral content is altered, which could favour rather than prevent subsequent microbial contamination. The microbial count depends on the hygienic status of the treatment system and the length of time during which the water is left to stand before it is used as drinking water. Further hygienic treatment (see sections 2.3 and 2.4) is necessary depending on the intended use.

On the other hand, the demineralization/partial desalination of water can also be an important pre-treatment process for subsequent water purification, especially in the case of water with a very high calcium concentration (so-called hard water). In such cases, it is recommended that demineralization be carried out before the water is autoclaved or before it is used in automatic drinking systems in order to prevent blockage of the drinking nipples.

Since demineralized water has a strong "drive" to take up minerals, it is highly aggressive in its effect on metals and alloys. The selected material for pipes and fittings must comply with the specifications for demineralized water. Partially desalinated water has a lower degree of aggressiveness in relation to the degree of desalination.

2.2.1. Reverse osmosis

Reverse osmosis is a process for the demineralization of water. It is not possible to produce sterile drinking water by means of reverse osmosis. A low microbial count always remains, even with an optimally functioning system. A systematic separation of the primary and secondary circuits is recommended, because otherwise there is a risk of contaminated water entering the clean secondary circuit from the primary loop. If the basic technical principles and service requirements of the system are not observed, the O-rings can become a weak point in the reverse osmosis system. Problems then arise in the contamination of downstream piping systems, either in the form of retrograde contamination or through "seepage water" from the primary loop. Usually there is first a colonization with Pseudomonas bacteria, which form a biofilm that in turn provides a good basis for pathogenic micro-organisms

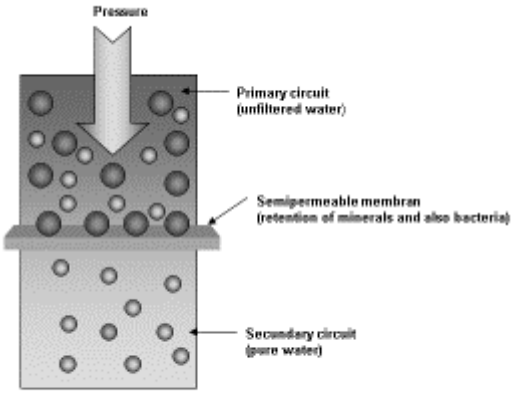

Abb. 1 Schematic diagram illustrating the principle of reverse osmosis

The main area of use for reverse osmosis is dialysis. It is strongly advised not to use reverse osmosis as sole method of hygienic water purification for drinking purposes in SPF housing facilities. The reverse osmosis should be combined with other water purification techniques,

e.g. with an ion-exchange system and a method for producing water that is sterile or has a low-microbial count (see 2.3, 2.4). Costs are incurred for several purification systems (Brandstetter, 1998a).

Conclusion: reverse osmosis is technical complex and can be used for the demineralization of water, but does not result in sterile drinking water. Therefore, the use of this technique should be reserved for combination with other methods, e.g. with heating or acidification.

2.2.2. Ion exchange

Various minerals are removed from drinking water using ion exchangers. Different water qualities can be produced depending on the ion-exchanger resin used - from partially desalinated water ("soft water") to fully desalinated water (FDW/demineralized water).

Cation exchangers are often used for softening purposes, removing Ca^+ and Mg^+ ions from drinking water. The method rests on the principle of exchanging Ca^+ and Mg^+ ions for Na^+ ions. This gives rise to so-called soft water with a higher proportion of bound Na+ ions than in drinking water.

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

Water is desalinated using ion-exchange embedded between ion-selective membranes. These membranes are anion permeable or cation permeable and act as a barrier to the main water flow, but allow the passage of ions. This means cations can pass through the cation permeable membrane (mostly H^+ as reactive group), but not anions. Accordingly, anions can pass through the anion permeable membrane (mostly OH- as reactive group), but not cations. This process may take place under the influence of an electrical field (e.g. also with water purification in special systems for laboratory requirements) or by applying pressure. The drinking water that is fed in continuously releases ions, which accumulate in the exchange resins and in concentrate channels. Their capacity determines the effectiveness of the system. The outcome of the exchange process is fully desalinated water (MerckMillipore).

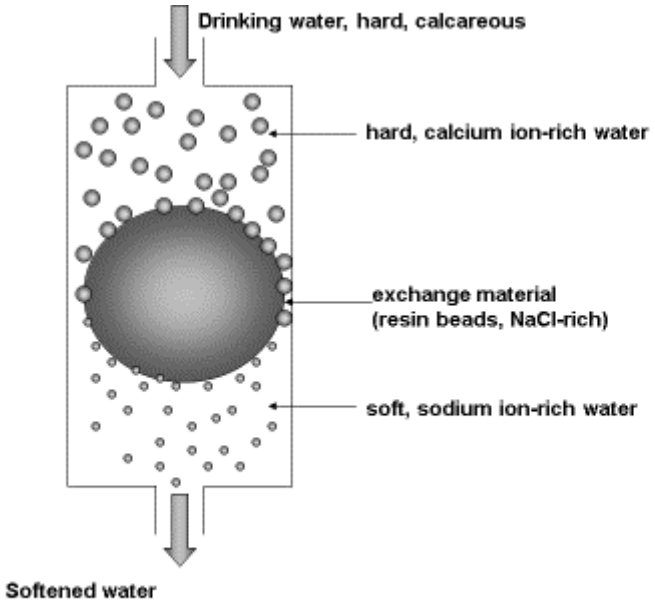

Fig. 2 Schematic diagram illustrating the principle of water softening on the basis of ion exchange

Conclusion: The production of partially or fully desalinated water is associated with additional costs. These water qualities are often present in animal housing facilities because they are also needed for other equipment (such as cage washing equipment and autoclaves). Only a minimal share of intrinsic costs would then be incurred for purification of drinking water. The use of demineralized or

partially desalinated water for drinking purposes essentially depends on the quality (“hardness”) of the drinking water and the further processes for hygienic treatment (e.g. heating) and provision of water (e.g. automatic drinkers). Use of this method does not necessarily result in sterile drinking water.

2.2.3. Distilled water

The use of distilled water is especially common where autoclaved water is used for drinking purposes. It offers the technical advantage, particularly with very hard mains water, that storage vessels and drinking bottles do not “fur up”. However, the water does not necessarily have a low microbial count. Even with this kind of water purification, the microbial count depends on the cleanliness of the purification system and the period of time during which the distilled water is left to stand in storage vessels until it is used as drinking water. Further hygienic treatment according to the intended use of the water is essential. For use in centralized drinkers, the regulations for piping and fittings must be observed in the same way as with demineralized water. No negative effects or signs of deficiency have been observed in animals, including their offspring, even with lifelong administration of distilled water. The mineral needs would appear to be sufficiently met with the feed in this case, as also in the case of mains water with a very low mineral content. The use of distilled water is associated with increased costs and only makes sense in special applications, e.g. when animals are housed in an isolator. Distilled water and water 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 of equal value to water demineralized using ion exchangers. It is costly and therefore only recommended for special applications (se also 2.4.1 Heat sterilization). The use of this method does not necessarily result in sterile drinking water.

2.3. Water with a low microbial count

In animal stocks where the microbial burden in the drinking water could (as described in 2.1) be a source of disturbance, while the use of sterilized water is too costly and not necessary, there are various methods available for treating the water.

2.3.1. Chlorination

Chlorination of the drinking water is a simple, low-cost method that has been tried and tested. It is by far the most commonly used. With this method, dosing pumps are used to add chlorine gas or other chlorine compounds (such as sodium hypochlorite or chlorinated starch with a high degree of purity) to the water, leading to a reaction with organic substances (manual addition of chlorine is also possible). The remaining residue or surplus of chlorine ions leads to disinfection and also sterilization of the water. Chlorine has a rapid microbicidal effect and, as a result of its gradual binding to particles contained in the water, a lasting effect in the water pipeline system.

Excessive chlorination can lead to unpleasant odours and irritation of mucous membranes in humans and animals (Thunert et al., 1975).

In commercial breeding, chlorination is frequently used for the purification of drinking water (usually in combination with other methods, e.g. acidification). Chlorination may be carried out using sodium hypochlorite. Experience here indicates that active chlorine becomes effective at a pH value of 5.0. The free chlorine concentration should amount to 6-8 ppm; in combination with acidification to pH 5.0, water that conforms to the requirements of SPF housing conditions can then already be obtained (Leblanc, 2002).

For housing mice, 15-20 ppm chlorine is sometimes recommended, although Bywater et al. (1977) already managed to keep the drinking bottles free of bacteria for 7 days with chlorine concentrations in the drinking water of 5 ppm upwards. Homberger et al. (1993) achieved freedom from Pseudomonas aeruginosa in mice (faecal evidence) with 6-8 ppm of active chlorine. The drinking bottles were changed once a week. With a more frequent change of bottles or an automatic drinking system connected to an automatic chlorine gas system, the chlorine concentration could also be lower. At the time of the bottle change, the chlorine concentration should never be less than 2 ppm.

Conclusion: The chlorination of drinking water is a very effective and low-cost method that is tried and trusted. It can be used with an acceptable degree of effort. Combination with other methods to increase the microbicidal effect should be reconsidered (acidification, filtration).

2.3.2. Acidification

Another possible method for treating water is acidification, which yields drinking water with a low microbial count. It does not achieve sterility! Almost all microbial species grow slowly at a pH of 2.0 to 3.0. A measurable increase only occurs after some time (about 3 to 5 days). The results of acidification, especially in preventing the spread of gram-negative species (particularly Pseudomonas aeruginosa) are good, although these microbes, too, are not killed.

Various acids may be added to drinking water (sulphuric acid or hydrochloric acid, Hall et al., 1980; peracetic acid, Juhr et al., 1978); hydrochloric acid is the most commonly used for acidification.

Assessments of the effect that acidification has on laboratory animals vary. Hall et al. (1980) found an effect on the microbial burden in the small intestine depending on the acid used. Acidification to pH 2.0 using sulphuric acid reduced the number of bacteria isolated from the terminal ileum more than acidification using hydrochloric acid. However, mice given acidified water generally showed significantly less bacterial growth after 6 weeks of treatment than mice given normal drinking water.

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

Clausing et al. (1989) recommend not reducing the pH below 3.0 (rat); at pH 2.0 they observed slight proteinuria with a reduced volume of urine.

Other authors did not find any signs of compromised health following the administration of acidified water (Tober-Meyer et al., 1981; rats, rabbits).

A further problem to be borne in mind with this method of water treatment is the selection of usable materials. One disadvantage is that many materials, such as drinking nipples and cages of galvanized metal, are not acid-resistant and tend to corrode. This necessitates the use of appropriate stainless steel or plastic materials. Copper, brass and most aluminium alloys cannot be used, because they corrode (AK KAB, 2013, chapter 9).

If bottles with stoppers are used, the leaching of minerals from the stoppers may be greater with deionized and acidified water than with untreated water. Kennedy et al. (1991) recommend plastic stoppers of red vinyl or silicone, which are more expensive than the usual black stoppers. The leaching of minerals from new stoppers is much higher than it is from used and repeatedly washed stoppers.

Combinations of acidification and other methods provide effective disinfection of the water. For example, the water may first be acidified to pH 5.0 using hydrochloric acid with the aid of dosing pumps, then concentrated with chlorine (6-8 ppm active chlorine) likewise with the aid of dosing pumps. A (usually automatic) readjustment of the pH is necessary. There are companies that offer this technique.

Conclusion: Acidification is a suitable method of obtained water with a reduced microbial count. It requires the use of materials resistant to corrosion and is therefore expensive in terms of follow-up costs. In combination with other methods (such as chlorination) it is possible to achieve sterility of water.

2.3.3. UV (ultraviolet) irradiation

Ultraviolet (UV) microbicidal irradiation is approved for the disinfection of drinking water. Using this method, UV lamps emit the disinfecting ultraviolet light in the flowing water. The necessary uniform irradiation can be achieved using a turbolator. UV light in the range of 200-280 nm exerts a microbicidal action, the maximum effect occurring at 260 nm. The maximum absorption of light by the nucleic acids of a microorganism’s genetic material also occurs at this wavelength, so that their DNA and RNA are altered by UV light. This results in microorganisms losing their ability to multiply. No long dwell times are needed for UV disinfection, because these processes take place in fractions of a second. The exposure dose at the end of the UV lamp life should amount to at least 40 mJ/cm³; this will result in a reduction of microbes by 105. The UV light emitted by the UV lamp should be below a wavelength of 240 nm in order to prevent the production of ozone and the formation of harmful by-products in the water to be disinfected (www.bwt.de).

The UV disinfection systems are stainless-steel flow-through reactors of compact structure that are simple to operate. Equipment should be rinsed (automatically as far as possible) while the water is stagnant. UV disinfection systems are used e.g. in the disinfection of drinking water for communal and private sectors and also in the beverages, food and pharmaceutical industries (www.bwt.de).

Publications from the 70s and 80s work on the assumption that a successful sterilization of drinking water cannot be accomplished with UV light (Thunert et al., 1975). The problems are seen to lie in the following factors: the microbicidal effect of the UV light is especially dependent on the quality of the water and the duration of exposure to and intensity of the light. If there are many organic substances and salts present in the water, the penetration of the light is only

minimal, and the light will fail to reach microbes that are embedded in particles of dirt or occur in colonies.

Conclusion: This method is recommended as an environmentally friendly technique for the disinfection of drinking water, which does not alter the water in any way. Whether or not sterility is achieved depends on the system and the prevailing conditions (mains network, quality of drinking water etc.). For SPF facilities, UV disinfection of the drinking water on its own does not guarantee adequate safety.

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_3) contains one oxygen atom more than (normal) oxygen (O_2). It is used as a disinfectant and oxidant and exerts a bactericidal, virucidal and fungicidal action. Ozone also oxidizes any organic and inorganic contaminants entering the water without the formation of disturbing reaction products. Pure oxygen occurs as residue (O_2); (www.bwt.de).

Treatment with ozone requires the use of a special system, which is usually associated with high acquisition costs. The principle of the systems consists in generating ozone from air by means of electricity. High-quality materials need to be used, such as housings of stainless steel and special glass (breakage resistance). The air supplied to the system must be dried and, if necessary, also purified by means of absorbers (or use of highly effective, macroporous molecular sieves). The regeneration of dryers affects the life of ozone generators. The heat arising with ozone generation is cooled down; with indirect cooling, no water can enter the high-voltage area even if the glass breaks.

The quantity of ozone generated can be adjusted to demand at any time. For safety reasons, ozone is produced and transported under negative pressure (vacuum). In special ozone water mixing systems, the ozone must be intensively mixed with the water to be treated (ensuring ozone solubility and avoiding losses of exhaust gas, www.bwt.de). The ozone can also be added to the water via so-called inoculation sites (Bieniek et al., 1981). A measure of both the microbicidal rate and the viability of microbes in the water is the redox value; as the redox potential increases, so too does the microbicidal effect. Bieniek et al. (1981) reported redox values between 580 and 780 to preserve bacteria-free water. In their experience, water containing ozone can disinfect the piping systems right up to the drinking nipples. Despite these possibilities, Bienek et al. (1981) recommend combination with another water purification method (e.g. acidification).

Residual ozone must be removed from the water, either using activated charcoal or catalysts or by means of UV treatment (Thunert et al., 1975; Bieniek, et al., 1981; www.bwt.de).

Conclusion: Not to be recommended for smaller animal housing facilities in view of the high cost. Use in combination with other methods is recommended.

2.4. Germ-free water

Gnotobiotic and germ-free animals may only be supplied with germ-free water. The use of germ-free water is likewise recommended for specific pathogen-free animals. For the sterilization of water, methods should be used that result in proven sterility of the water.

2.4.1. Heat sterilization

For the sterilization of drinking water in the autoclave, the following points must be borne in mind:

- Drinking water can be sterilized in the drinking bottles.
- If the bottles are autoclaved with the drinking caps fitted, then - depending on the caps used (size of aperture) - the caps may come off or the bottles be damaged as a result of the prevailing pressure conditions during sterilization. It is therefore recommended that the water be autoclaved in the bottles without caps and to fit the likewise sterilized caps only after sterilization.
- Some of the salts dissolved in drinking water precipitate out on heating (carbonates). The precipitated “calcium flakes” can block the apertures in the drinking caps. The water should therefore be softened before sterilization; demineralized or distilled water are also very suitable. An exception of course is very soft water.
- The material of the drinking bottles used may affect the sterilization temperature. Macrolon bottles can be autoclaved at not more than 120-125°C according to manufacturer’s instructions.
  Since the attainment of sterility is dependent on pressure, time and temperature, sterile material can also be obtained at temperatures below 121°C varying the parameters. Experience shows that the wear and tear on Macrolon can be markedly reduced at lower temperatures. Macrolon bottles are therefore often autoclaved at temperatures of only 116-118°C with longer exposure times. Good experience has been obtained for the sterilization of drinking water in Macrolon bottles at 116°C with an exposure time of 40 minutes and at 118°C with an exposure time of 20 minutes. These data relate to a particular type of equipment (MMM Vakulab PL) and must be verified for any other equipment (see also GV-SOLAS, Committee for Nutrition of Laboratory Animals, Yellow Booklet “Hygienic treatment methods for feed, steam sterilization of feed in the autoclave”, 1998).
- When sterilizing drinking water in bottles, due account must be taken of the relatively long preheating time and above all the long-lasting cooling process after sterilization depending on the total volume of liquid in the autoclave. Relatively large quantities of water (200 litres or more) are therefore best autoclaved overnight. Of course, the total duration of the programme also depends on the equipment specifications (e.g. active cooling systems and procedures) of the steam sterilizer itself and the in-house steaming and cooling capacity. Before removing the sterilized material from the steam sterilizer, it is absolutely essential to make sure there is an adequate cooling of the system. The safety recooling temperature must be less than 80°C before the system can be opened, otherwise superheating may occur (Brandstetter, 1998b).

Conclusion: This type of water treatment is recommended with animal housing in isolators, especially since an autoclave is generally needed for it. In view of the length of time it takes for the sterilization process, this method can only be used for limited quantities of liquid and is not suitable for automatic drinking systems.

2.4.2. Sterile filtration

Filters with the smallest possible mesh size are used for the filter devices. A filter mesh size of 0.2 µm is needed to achieve sterility (Thunert et al., 1983; Brandstetter, 1998c).

A prefilter system is recommended to prevent rapid contamination of the filters. There are several upstream stages depending on the quality of the water. As a result, most particles (inorganic and organic, especially bacteria) up to a size of 2 µm are separated off without burdening the sterile filter (e.g. Pall or Hosch filter). In addition, water that has been softened or fully desalinated by means of ion exchangers may also be used (see 2.2.2).

More recent filter systems can be mounted directly at the water sampling point and prevent microbes growing through the filter membrane through the use of special technologies (Warncke, 1998).

Filters are only effective if they are carefully and correctly maintained. If they are not, they tend to cause damage, because they then become a reservoir for microbes (note manufacturer's instructions).

It is a prerequisite for the successful use of filter devices that the filters and piping systems upstream of the filters are reliably sterilized. These piping systems should be kept as short as possible. With continuous use of water, the filters should be sterilized at least twice a week. With non-continuous use, the filter should be sterilized (in the autoclave) each time before use. Depending on the filter type, it may be autoclaved between 3 and 30 times, after which the filter must be tested for its functionality each time (special tests available, filter sterilization and tests performed according to manufacturer's instructions). Filters are not suitable for discontinuous use without constant checks.

Besides a check on the filters, it is also recommended to test the drinking water at regular intervals (weekly to fortnightly) for sterility (Raynor et al., 1982).

Because of the fine mesh filters, the water has to pass through the filter system with corresponding pressure. All filters consequently undergo an increasing pressure drop in the mains network (Thunert, 1975). To attain a sufficiently high water pressure, it is possible (depending on the pressure in the mains network for drinking water) that an additional pressure boosting system may be necessary (Brandstetter, 1998c).

Conclusion: Sterile filtration is only useful and effective if the filters are located at the end of the piping system. A system of this kind is maintenance-intensive, but safe if regularly maintained.

2.4.3. Combined demineralization/decontamination procedures

The combination of water softening (ion exchange / reverse osmosis) and subsequent sterilization by heating to 134°C followed by acidification to pH 2.7 with HCl is an efficient method for relatively large systems. However, this type of water purification requires a high technical investment (tanks and dosing systems), the avoidance of long standing times in the pipes (e.g. automatic flushing at night) and regular monitoring of parameters.

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

Conclusion: The choice of methods to be combined for relatively large animal facilities depends on the resources available (technical and financial) and on the quality of water required

3. Method of administering water

Water is administered either via drinking vessels that can be individually operated or via automatic drinking systems. Aside from the technical adaptation of the vessels to the housing system, the only condition is that the animals must have free access to the watering facility. If the water consumption has to be registered for the purposes of the experiment, measuring vessels must be used.

3.1. Drinking vessels

For drinking vessels that have to be frequently filled individually by hand, buckets and bowls (for large laboratory animals such as sheep, goat, pig, dog or cat) or bottles may be used. Drinking bottles are suitable for almost all laboratory animals (up to about 3 kg bodyweight); there are differences in the design of the drinking nipples.

Bottles with short and/or long nipples are used for rats, guinea pigs and mice. Long nipples have the advantage that newly weaned animals can also access the water. Short nipples are more suitable for larger animals (rats), because they are not unintentionally touched by the animals so often (water loss). The fill level of the bottles must be regularly checked. This provides the experimenter with information on drinking behaviour (wellbeing of the animals) and on the functionality of the bottles. If they are not properly closed, and also if the pressure in the animal room fluctuates, it is possible that the water will leak out. Wet young mice then very quickly suffer hypothermia, fall sick and die. But it can also happen that animals die of thirst despite the drinking bottle being full. New drinking nipples may have sharp edges, which hinder the animals from drinking, or the water does not flow because the nipples have not been properly degreased. Bedding material and residues of food can block the aperture in the nipple. If there is no air bubble in a full bottle, smaller mice have difficulty getting water out of the bottle.

It is recommended that the bottles and caps be changed and cleaned at least once a week.

3.2. Automatic drinking systems

Automatic self-drinkers can be used for all laboratory animal species. They can be provided in the form of drinking bowls or nipple drinkers depending on the species.

The nipples must be regularly checked for their functionality. Nipples that constantly drip can fill the plastic cages with water overnight lead to the death of the animals by drowning. Cleaning the nipples of an automatic drinking system is hardly possible while it is in operation. Cleaning therefore has to wait until the general cleaning of the room or shelf.

3.3. Wet feed

In special situations, the provision of wet feed has proved successful. For example, it can help newly weaned mice to take up water if wet feed (soaked pellets) is additionally provided, e.g. in a Petri dish or directly in the cage bedding. It must be borne in mind that mould may grow if any residues of the feed are not removed in good time.

It is likewise recommended that wet material be added when animals are transported over the course of several hours. The simplest method is to soak the animal feed (pellets with a low microbial count or sterile) overnight in a vessel and then to place them in the transport container.

Some breeders use wet feed sterilized in plastic covers (sausage-shaped) or gelatins heat-sealed in plastic, which is also commercially available as “solid drink”. This plastic cover has to be torn open when it is used to enable the animals to access the feed.

It is advisable not to use raw potatoes, carrots or apples for SPF animals. An alternative is to provide potatoes sterilized in preserve jars (Hagelschuer, 1998).

Self-prepared gelatines (water + gelatines, soak, heat to 70°C and then cool) is also a suitable way of supplying animals during long journeys (Thomae, 2001).

4. Recommendations from the committee

For the supply of water to conventional animals, the quality of the drinking water delivered from supply systems in accordance with the drinking water ordinance is generally sufficient. For the housing of SPF animals in a breeding facility or for certain experiments (e.g. measurements of mineral input) the drinking water must be treated to demineralize it and/or reduce/eliminate the microbial burden.

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

With all methods of water treatment, the drinking vessels must regularly cleaned or replaced because they become contaminated with the oral microflora of the animals, which come into contact with the vessels when the animals drink from them! (Tillmann, 2015).

The following methods are recommended depending on the engineering provided (e.g. pipes) and the financial resources available:

To achieve a low microbial count in the water:

1. Chlorination (cheapest method)
2. Acidification to pH 2.5 – 3.0 (more expensive, because only certain materials are acid-resistant)
3. Combination of several methods (often very expensive)

To achieve sterility of the water:

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

For the demineralization of water:

Ion exchange

Before setting up the water supply system, it is recommended that water purification systems in other laboratory animal facilities be looked at so as to obtain manufacturers' specifications and pick up practical tips. Based on the knowledge of these methods, the water purification should be tailored to individual needs together with the manufacturer.

5. Annex 1: Applicable legal regulations on the quality of drinking water quality, January 2016

The ordinance on the quality of water for human consumption (Drinking Water Ordinance [TrinkwV] in the version published on 2 August 2013 [German Federal Law Gazette BGBl. I p. 2977], amended by Article 4 Paragraph 22 of the Act on 7 August 2013 [BGBl. I p. 3154]), regulates the requirements, reference values and limits as well as permitted deviations in Germany. The hygienic parameters to be monitored extend to:

microbiological parameters: not more than 100 germs may be detected in 1 ml water and, in accordance with defined threshold values, no Escherichia coli, no coliform bacteria and no Enterococci may be contained in 100 ml water (Annex 1 to § 5 Para. 2 and 3, BGBl. I 2013, 2992, TrinkwV).

and

chemical parameters: permitted threshold values of chemical parameters in the distribution network of a house installation are listed in Annex 2 (to § 6 Para 2; BGBl. I 2013, 2993 - 2995) of TrinkwV.

The ordinance permits deviations from the norm if e.g. geographical peculiarities result in a change of water quality and the deviations are not harmful to human health. In emergencies, time-limited deviations are possible (TrinkwV, 3. Section, § 12).

It is therefore always advisable to obtain information about water quality before the start of an experiment

6. Literature

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