Specialist information

from the Committee for Animal Welfare Officers (GV-SOLAS)

Food and water deprivation in laboratory animals

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Contents

A. General part

1. Preliminary remarks

The following explanations are limited confined to laboratory animal species for which literature data is available on the physiological effects of temporary water or food deprivation and for which objectifiable assessments of stress can therefore be made. All information here applies only to laboratory animals whose physiological requirement for food and water is species-typical. Stress levels for strains or lines with specific nutritional requirements must be assessed separately.

2. General principles

It follows from the minimum legal requirements for the housing of laboratory animals (§ 2 TierSchG, § 1 TierSchVersV) that a regular supply of suitable food and water must be guaranteed. Access to food and water may therefore only be restricted or prevented if this is essential to achieve the purpose of the experiment. This only applies if other, gentler methods cannot be used and the extent of feed and/or water deprivation is necessary to achieve the intended purpose. The withdrawal of feed and/or water in an animal experiment is subject to approval according to § 7 TierSchG and must be documented in the animal experiment records.

Any deprivation must be kept as short as possible and restricted to the absolute minimum. During deprivation, the condition of the animals must be checked frequently enough to detect any deterioration in their health. If the intervention endpoints defined in the application for approval of the animal experiment are reached, appropriate measures must be taken immediately. If there is any doubt, the animal welfare officer must be consulted.

3. Terms

Feeding/watering *ad libitum*:	Constant supply of food and water is freely available.
Rationed feeding:	The planned total quantity of feed is dispensed in partial amounts at certain times taking into account the requirements of the individual animal (e.g. via computer-controlled automats).
Pair feeding:	Animals in a control group receive the quantity of feed previously consumed within a defined period (usually 24 hours) by animals from the study group (Weiß et al. 2009, Güttner et al. 1993).
Deprivation:	Is used here as a generic term for withdrawal or restriction of food and/or water in an animal experiment.
Withdrawal of food/water:	Temporary withholding of food or water in an animal experiment.
Food restriction:	Restricted supply of food as measured by the daily requirement. In the case of quantitative restriction, the amount of food and/or access time is limited, and in the case of qualitative restriction, the composition of the ingredients is modified.
Water restriction:	Restricted supply of fluids as measured by the daily requirement.
Intervention point:	The point when unacceptably high levels of stress occur in the animal, necessitating immediate veterinary intervention.
Standard animal husbandry:	Laboratory animal husbandry in accordance with § 2 TierSchG and § 1 TierSchVersV.

4. Research projects that can lead to food and/or water deprivation

4.1. Withdrawal of food and/or water

a. Food deprivation during anaesthesia and surgical procedures is used to prevent vomiting and regurgitation (ruminants) with the consequence of possible food aspiration as well as the risk of suffocation and pneumonia. In rodents and rabbits, preoperative withdrawal of water and food is not advisable in view of the risk of hypoglycaemia and acidosis developing and also the fact that these animals cannot vomit (Erhardt et al. 2012, GV-SOLAS 2012a). In addition, even deprivation periods of up to 48 hours do not lead to complete emptying of the gastrointestinal tract in most of the laboratory animal species used (Erhardt et al. 2012).

   Further information on the withdrawal of food and water in relation to anaesthesia in laboratory animals can be found in the specialist information on “Food withdrawal in the context of anaesthesia in laboratory animals” from the GV-SOLAS Committee for Anaesthesia (GV-SOLAS, 2012a).

b. In metabolic and kinetic studies, food deprivation may be used to achieve standardized baseline values and to avoid extreme variation of results.

c. Withdrawal of food in “pair feeding” or similar experimental feeding regimens can serve to identify experiment-related effects, e.g. a refusal by the laboratory animals to feed (anorexia). Pair feeding can also be used to determine whether reduced weight gain is caused by reduced food intake or by a change in metabolism.

d. The withdrawal of food and/or water can also be used to induce states of hunger or thirst, if these are necessary for the performance of the experiment or if these states are the actual objective of the study.

e. In the case of behavioural studies in neurophysiological investigations, prior withdrawal of food or water may be necessary when it comes to positive reinforcement in order to give the animals the incentive to seek the reward. It must be established beforehand whether or not the motivation or the learning success of the animals can be achieved by other measures, such as the administration of particularly coveted food without prior withdrawal of food (Prescott et al. 2010).

4.2. Restriction of food and/or water

a. Nutritional or metabolic studies may require changes in both the quantitative and the qualitative supply of feed.

b. In behavioural studies and learning research involving the use of feed or fluids for positive reinforcement, restrictions of food or water may occasionally occur if the animal is unable to meet its daily requirements through the rewards.

c. Evidence suggesting that sustained calorie restriction can lead to a significant increase in the lifespan of laboratory animals (e.g. Heilbronn and Ravussin 2003) is not a suitable justification for food restriction in a research project, especially since this can also have the opposite effect, depending on strain and age of the animals (e.g. Forster et al. 2003).

5. Advice on experiment-related use of food and/or water deprivation

5.1. General information

a. Body weight, water consumption and the amount of food consumed by the animals must be regularly monitored and documented. The drinking water must be checked daily and body weight at least weekly or as much as daily, if necessary, to make sure any deterioration in health is detected early enough.

b. Given an adequate period of acclimatization, rodents and many other animal species adapt well to a once or twice-daily feeding regimen (Gärtner 2001, Rowland 2007). However, the timing of deprivation is a major factor to consider because food intake in many animal species is subject to a circadian rhythm. A short deprivation period that does not take the circadian rhythm into account can therefore be just as stressful as a 24-hour deprivation period (Rowland 2007). Feeding also serves as an activity function that differs in importance from one species to another.

c. The animals' food requirements are heavily influenced by age, housing conditions, differences between strains and other factors. When it comes to requirements for drinking water, the data in the literature vary even more widely. Water intake is not substantially influenced only by the physiological need for fluids, but also by social variables (hierarchy-dependent water intake, play behaviour etc.). The need for feed and fluids should therefore be carefully established for each animal before an experiment (NRC 2003, p. 55/56).

d. The absolute and/or relative weight loss of the laboratory animals resulting from food and/or water deprivation must be determined on the basis of an objective comparison. The basis may be the published growth curve for the species, breed, line, or strain in question, for which the body weight in adults before the start of the study and the food or water intake of non-deprived control animals or also of pair-fed control animals are suitable variables. It also has to be borne in mind that further experimental treatments, aside from deprivation, may result in additional weight losses. If the defined intervention point is reached, supplemental feeding and watering must be initiated for the animal immediately.

e. The following intervention points must be specified in the protocol:

- Level of weight loss:
  This is usually a weight loss of more than 20% of the reference body weight (Morton and Griffiths 1985, Foltz and Ullman-Cullere 1999, OECD 2000), measured in comparison with the control data defined under d);
- Dehydration (skin tent time or other parameters);
- Extent of species-specific deviations in behaviour, in morphological and physiological characteristics (see below).

An accurate appraisal of the wellbeing or stress state of an animal cannot be made on the basis of a single physiological characteristic, but only through the regular assessment of different characteristics (NRC 2003, p. 60).

f. In the case of water restriction, it must be borne in mind that drawing an analogy between human thirst and an animal's requirement for water is only possible to a very limited extent. Humans, for example, respond to 24-hour water deprivation with a considerably greater increase in plasma osmolarity than the rat or dog (Toth and Gardiner 2000). Animals species adapted to arid habitats and hence naturally to conditions where water is not always readily available are less stressed by water restrictions than species from non-arid habitats (Lindburg 1977).

g. Food and water intake are closely correlated (Silver et al. 1991, Verplanck and Hayes 1953). Even with unlimited access to dry feed, water deprivation leads to a progressive decline in food intake. To ensure that food intake is adequate, therefore, water should be available for a sufficient period of time (Maren and Fanselow 1998). In most laboratory animal species and in humans, a period of 20-30 minutes is sufficient to normalize haematological changes and the feeling of thirst after 24 hours of water deprivation (Toth and Gardiner 2000, Lesser 2003).

h. Special requirements with regard to feeding and the storage of feed must be defined in the protocol and should be agreed with the animal technicians.

5.2. Behavioural studies and conditioning experiments

In behavioural conditioning studies, it is preferable to use positive reinforcement by providing food the animals prefer rather than negative reinforcement by withdrawing food, if the intended conditioning can be achieved in this way. If a preferred food is used as a reward, then food restriction is unnecessary in many cases. This approach is also preferable for reasons of quality assurance because food deprivation can have a negative impact on learning behaviour, behaviour in the open-field test and the stress reaction (Heiderstadt et al. 2000, Sherwin et al. 2003). A prolonged series of experiments requires repeated checks to establish whether the length of food deprivation can be reduced without jeopardizing the success of the experiments, as suggested by various studies (NIMH 2002).

If food is used as positive reinforcement, it is essential to ensure that the nutritional value of the freely available food and the food provided as a “reward” guarantees a healthy nutritional status of the animals, i.e. does not lead either to malnutrition or to overweight (GV-SOLAS 2012b).

If milk and fruit juices are used as positive reinforcement in long-term behavioural conditioning studies, it must be borne in mind that they spoil easily, so they must be frequently replaced and require more time and effort in the cleaning of equipment (NRC 2003, p. 60).

Depending on the animal species, the behavioural task and the requirements defined in the study protocol, food and/or water deprivation may be unavoidable. When it comes to the reasoning required to justify this step, the following points must be borne in mind:

a. The current literature must be reviewed to establish whether food/fluid deprivation cannot be replaced by alternative methods or conducted in a manner that is gentler for the animal.

b. If food/fluid deprivation has to be used in the experiment, the methodology and scientific justification must be described in the application for approval of the animal experiment.

c. Food/fluid deprivation must not exceed what is required to achieve the objective of the experiment and must be described with reference to measurable characteristics (intervention points, see 5.1.) and/or the period of deprivation within a 24-hour day. As a rule, the animals must be weighed at least once a week in order to keep a regular record of food/water consumption and of body weight; if new study protocols are introduced, a daily measurement of water and food intake, body weight and hydration status may be necessary. Here, the quantities not consumed by the animals, but scattered or “squandered” (e.g. parts of food pellets in the case of rats or drinking water with guinea pigs or pigs) must be recorded or estimated and deducted from the total consumption measured.

d. To define deprivation, it is necessary to know the quantities of food and fluids that correspond to the normal requirements for the sustenance of the animals. Here, the individual needs of the animals (e.g. growth phase, pregnancy, lactation, age and health) and the aim of the study must also be taken into account. In most animal species, food and water intake follow a circadian rhythm, so nocturnal animals show the most intensive intake at night. Deprivation during this phase thus also leads to greater stress. For the level of food intake, the time of day is more important than the energy deficit or the length of deprivation (Porzig and Sambraus 1991).

B. Special part

6. Species-specific severity levels of food and/or water deprivation (not deprivation in the context of anaesthesia)

Depending on the duration and the laboratory animal species, the withdrawal or restriction of food and/or water expose the animals to differing degrees of stress, which manifests itself in various physiological and/or behavioural ways and causes hunger, thirst and malnutrition. It can result in metabolic and neurohormonal disorders, electrolyte imbalance, hypovolaemia, hypotension, hypertension, and gastrointestinal motility disorders, as well as affecting thermoregulation, circadian rhythm, and behaviour (Toth and Gardiner 2000, Tucci et al. 2006, Rowland 2007). As long as the body has not adapted to the restriction, signs of stress will occur that are associated with negative effects on wellbeing and can therefore be described as “suffering” (Lorz and Metzger 2008). Animals may adapt to restrictions within a few days, but it can also take several weeks (Toth and Gardiner 2000, Rowland 2007).

Estimates are given here for severity levels during periods of deprivation in frequently used laboratory animal species for which relevant data are available in the literature. Experimental severity levels beyond those related to deprivation are of course not covered by these estimates and must be additionally taken into account when estimating overall severity.

Needless to say, brief periods of water or food withdrawal cannot be regarded as severe, because animals also spend time naturally without food and water intake. However, since no unequivocal data can be found in the literature reliably demonstrating the period of time up to which a withdrawal of food or water cannot be regarded as a burden to the animal, no such time periods are indicated here. They should be estimated for each individual research question after consideration of all the various conditions (species, strain, circadian rhythm etc.), because it has to be assumed that species-specific tolerance levels exist for time-limited phases without access to food and water that are physiologically harmless.

As already mentioned, the following information is based on the species-appropriate physiological nutrition requirements of adult animals of the laboratory animal species concerned. Some breeds, strains or lines may have specific needs that requires a separate appraisal of severity. The same applies to non-adult animals as well.

Severity is classified as “mild”, “moderate” and “severe” according to Directive 2010/63/EU and Germany’s ordinance on laboratory animal welfare (*TierSchVersV*). A severity level greater than “severe” within the meaning of § 25 TierSchVersV was not appraised and must assessed additionally if animal experiments are carried out that lead to considerable and probably longer-lasting or recurring pain or suffering in the animals used.

6.1. Mouse (*Mus musculus*)

6.1.1. Species-specific information

Mice satisfy most (about 75%) of their daily food requirement during the activity or dark phase (Wiepkema et al. 1966, Kurokawa et al. 2000, Rowland 2007, Jensen et al. 2013). Since rodents also combine about 70–90% of their daily water intake with their food intake, the water intake also follows this circadian rhythm (Silver et al. 1991). Effects of food or water deprivation that lasts for less than 24 hours therefore depend very heavily on the time of day when this deprivation occurs. With increasing age, both the total volume of water intake and also the marked circadian rhythm decline, so by the time mice reach the age of 24 months nocturnal water intake only accounts for about 45% of their total daily water consumption (Silver et al. 1991).

The frequency of food intake in mice is markedly influenced by external factors such as food accessibility and consistency (e.g. liquid or pelleted feed) and may lie between 2 and 50 feeds per activity phase. It can thus be assumed that the point at which signs of stress occur following several hours of food deprivation will vary (Rowland 2007).

While the amount of food consumed within one hour after a one to six-hour food and water deprivation was similar to the amount consumed by control animals provided with feed *ad libitum*, the amount of food consumed within one hour after a nine-hour period of food deprivation was less than 50% of the control quantity with *ad libitum* feeding (Karami et al. 2006).

With combined food and water deprivation, mice showed a stress response in the hot plate test after only 24 hours in the form of significantly delayed pain reactions. By contrast, this response did not occur until after 48 hours with food deprivation alone and not until 72 hours had elapsed with water deprivation alone (Konecka et al. 1985).

It is also a natural feature of mouse behaviour that these animals go through periods of several hours in which they consume neither food nor water, without this leading to stress for the animals. **Therefore, not every brief period of food or water deprivation can be regarded as essentially stressful.**

6.1.2. Food deprivation

When classifying the severity of food deprivation, various aspects must be considered, such as the sex and age of the animals, genetic differences between strains, individual differences in the number of daily feeds or also the activity phase with short-lasting food deprivation (Rowland 2007, Xu et al. 2012, Jensen et al. 2013). For this reason, general assessments as to the degree of severity in relation to the duration of food deprivation can only be made to a limited extent. One of the few objective criteria suitable for the assessment of severity therefore is particularly the reduction or increase in body weight observed during food deprivation. The following figures thus serve only as reference points; the actual degree of severity must be verified through the regular monitoring of body weight.

The administration of 10% glucose in the drinking water, which does not lead to weight gain in mice that are fed *ad libitum*, can compensate for losses of body weight with food deprivation lasting several days (Zammaretti et al. 2001).

a) Mild

The severity of food deprivation in mice lasting up to 12 hours both in the light phase and in the dark phase or a weight loss of up to 5% is regarded as mild (Morton and Griffiths 1985).

b) Moderate

The severity of food deprivation lasting between 12 and 24 hours, which includes the dark phase, or a weight loss of between 5 and 20% must be considered moderate (Morton and Griffiths 1985, FELASA 1994, Moyal 1999).

c) Severe

The severity of food deprivation lasting more than 24 hours or a weight loss of more than 20% must be classified as severe (Morton and Griffiths 1985).

6.1.3. Water deprivation

The average daily water intake in mice amounts to about 25% of their body weight – subject to strain-specific differences (Rowland 2007).

The differences between strains of mice means there are also major differences in their adaptability to water deprivation. Extensive literature data on the consequences of water deprivation in mice are provided by Lesser (2003). In general, the first reactions of the body to water deprivation appear after 12 hours in the form of a reduced body weight, increased haematocrit and changes in osmolality and serum protein concentration. An increase in urine osmolality and a decrease in urine volume are observed with water deprivation lasting more than 24 hours (Lesser 2003).

If weight loss is considered as a severity parameter, the differences already mentioned in relation to strain, age, feed quality and also sex lead to very different results.

a) Mild

The severity of water deprivation for mice lasting up to 12 hours or weight loss of up to 5% is regarded as mild (FSVO 2016).

b) Moderate

Weight loss figures with 24-hour water deprivation vary from 6.7% (Rowland 2007) to 15% (Lesser 2003), whereas a 48-hour period of water deprivation is accompanied by weight loss of 15 to 20% (Lesser 2003, Bekkevold et al. 2013), so the severity of water deprivation lasting 12-24 hours can be regarded as moderate (FSVO 2016).

c) Severe

Even if weight losses of less than 20% are observed, water deprivation lasting more than 24 hours leads to physiological changes that must be classed as severe (Bekkevold et al. 2013; FSVO 2016).

6.2. Rat (*Rattus norvegicus*)

6.2.1. Species-specific information

In rats, as also in other mammals, there is a direct correlation between water intake and food intake. After food deprivation lasting between 48 and 60 hours, for example, water intake falls to about 30% of the control value, and food intake falls to about 20% of the control value after water deprivation lasting between 48 and 60 hours (Dicker and Nunn 1957, Kiss et al. 1994, Combet et al. 2008). While food intake falls immediately and continuously when water is withdrawn, water intake only falls after 48 hours of food deprivation (Armstrong et al. 1980).

In their natural habitat, there are phases when rats will go several hours without food or water and not suffer as a result. **Therefore, not every brief period of food or water deprivation can be regarded as essentially stressful.**

6.2.2. Food deprivation

Food intake frequency and intervals in the rat depend on the diurnal phase (Gärtner 2001). While 60-85% of the daily ration of food in the active dark period is consumed at intervals of not more than two hours, six to nine hours may pass in the (inactive) light period until the next feed (Rowland 2007). As a storage sac, the forestomach (*pars proventricularis*) in the rat provides food reserves for several hours. Unlike in dogs and humans, this leads to a constant postprandial blood glucose level lasting six or more hours (Gärtner 2001). Rats can adapt to a twice daily feed within two or three days and compensate for this with an increase in food intake per meal (Gärtner 2001).

Food deprivation in rats lasting several days increases the physiological day/night difference in body temperature, causing the body temperature to fall steadily during the light phase, while it remains constant in the dark phase (Yoda et al. 2000). Food deprivation also reduces anxiety-related behaviour, such as the acoustic startle response (Maniscalco et al. 2015), and encourages exploratory behaviour, e.g. in the open-field test (Heiderstadt et al. 2000). Based on the metabolic response to prolonged food deprivation, three phases can be distinguished, similar to those in humans. The early phase covers the first 24 to 48 hours. In the middle phase (2-3 days), fat reserves are mainly used for energy production (about 80%) and the body’s own proteins are initially supplied only to a mild extent (about 20%). Only in the late phase proteins are increasingly used for energy production (Goodman and Ruderman 1980, Goodman et al. 1980, Belkhou et al. 1991, Bertile et al. 2003). In the late phase, there is also a change in the pattern of diurnal activity, in which sleep phases are markedly reduced and wake phases correspondingly increased (Dewasmes et al. 1989). Weight losses, which occur with food deprivation of 48 hours or more, are not fully compensated for in most animals even after a return to *ad libitum* feeding (Armstrong et al. 1980).

The actual degrees of severity of food deprivation in rats may differ depending on the strain, sex or age of the animals. Pilot experiments with planned food deprivation can improve the assessment of the severity (Dietze et al. 2016).

a) Mild

According to Annex VIII, Section III of Directive 2010/63/EU, the severity of food deprivation lasting up to 24 hours in adult rats can be regarded as mild.

b) Moderate

The severity of food deprivation lasting between 24 hours and 48 hours is classified as moderate according to Annex VIII, Section III of Directive 2010/63/EU.

c) Severe

Food deprivation lasting more than 48 hours leads to a weight loss of between 15 and 25% depending on the age and strain of the animals (Gianotti et al. 1998, Kanayama and Liddle 1991, El Fazaa et al. 2000). It can also result in gastric ulcerations (Paré and Temple 1973, Jaffe and Desiderato 1978) and increased apoptosis in the intestinal epithelium (Ito et al. 2010). Food deprivation lasting more than 48 hours must be classed as severe.

6.2.3. Water deprivation

In rats, about 80-90% of the daily water intake occurs during the dark phase (Ang et al. 2000).

Access to drinking water for only 15 minutes a day does not lead to either behavioural changes or to an increase in serum corticosterone values (Heiderstadt et al. 2000). Compared with *ad libitum* access to drinking water, water deprivation lasting 7, 14 or 21 hours a day over a period of three months does not lead to negative effects on morphology, haematology or clinical chemistry apart from temporary weight loss (Hughes et al. 1994).

After 24 hours of water deprivation, plasma fatty acid concentrations are already markedly increased. This indicates that, with energy metabolism remaining unchanged, fats are increasingly used as a source of energy (Hohenegger et al. 1986).

Weight losses that occur as a result of water deprivation are usually completely compensated for within two weeks of returning to *ad libitum* access to water (Armstrong et al. 1980). Further extensive literature data on the consequences of water deprivation in rats can be found in Lesser (2003).

a) Mild

Although a 5-10% weight loss already occurs with 12 hours of water deprivation, the relevant laboratory parameters indicate that the severity of water deprivation lasting up to 24 hours in rats is mild (Lesser 2003, Rowland 2007).

b) Moderate

Water deprivation lasting up to 48 hours leads to a weight loss of about 15%, and relevant laboratory parameters also show marked or maximum deviations from normal values. In addition, the withdrawal of water for up to 48 hours causes an increase in plasma corticosterone concentrations by up to 100%, which is seen as a sign of chronic stress (Hohenegger et al. 1986, Brooks et al. 1997, Ulrich-Lai and Engeland 2002). The severity of water deprivation for between 24 and 48 hours must be considered as moderate suffering.

c) Severe

Water deprivation lasting more than 48 hours must therefore be classed as severe.

6.3. Rabbit (*Oryctolagus cuniculus*)

6.3.1. Species-specific information

All domestic rabbits are descended from wild rabbits of the subspecies *Oryctolagus cuniculus*, whose natural habitat is in the northeast of Spain and the south of France. If the water content of food amounts to at least 65%, wild rabbits can survive entirely without water intake presumably as an adaptation to their original habitat. In dry periods, however, when the water content of food falls to 10-15%, they are dependent on other sources of water (Cooke 1982).

With an *ad libitum* supply, food, and water intake even under laboratory conditions are inextricably linked, water intake showing a linear correlation with the amount of dry matter consumed (Cizek 1961). Therefore, food and water intake cannot be monitored separately from one another by restricting intake. Both time-limited and quantitative restriction of water leads directly to correspondingly reduced food intake (Denton et al. 1985, Verdelhan et al. 2004). When food is withdrawn, on the other hand, rabbits substantially increase their water intake (by about 650%; Brewer and Cruise 1994).

Weight loss with food deprivation does not appear suitable as the only criterion for the assessment of severity in rabbits, because the weight reduction remains well below 20% even after several days of food deprivation (e.g. 14% after 6 days, Weber and Reidy 2012). The slow weight loss following the start of food deprivation is also matched by an extremely delayed weight gain following the return to *ad libitum* feeding. This must be taken into account when the severity of food deprivation.

Another factor to consider is that not every brief period of food or water deprivation can be regarded as essentially stressful.

6.3.2. Food deprivation

Conditioning experiments using food for positive reinforcement cannot be recommended in rabbits, because the food deprivation that is necessary before supplies of food are accepted as a positive reinforcer would have to last for too long a period (Rubin and Brown 1969).

a) Mild

The severity of food deprivation for up to 24 hours is assessed as mild.

b) Moderate

Based on observations of lipid metabolism in rabbits subject to the complete withdrawal of food, the severity of food deprivation lasting between 24 and 48 hours is assessed as moderate (Reidy and Weber 2004, Weber and Reidy 2012).

c) Severe

The withdrawal of food for more than 48 hours is classed as severe even if the resulting weight loss is less than 15%. (Reidy and Weber 2004, Weber and Reidy 2012).

6.3.3. Water deprivation

Water deprivation also leads to a reduction in food intake (Ben Rayana et al. 2008) and the severity of water deprivation must therefore be regarded as greater than that of food deprivation alone. After three days of water deprivation, the animals practically cease feeding altogether (Brewer and Cruise 1994). Unlike food deprivation, however, water deprivation is suitable for conditioning experiments, because rabbits already show reliable behavioural reactions after 22 hours of water deprivation when they receive water as a positive reinforcer (Rubin and Brown 1969).

a) Mild

The severity of food deprivation for up to 12 hours is assessed as mild.

b) Moderate

The severity of water deprivation for between 12 and 24 hours is regarded as moderate (Kallaras et al. 2004).

c) Severe

The withdrawal of water for more than 24 hours is classed as severe (McKinley et al. 1983; Islam et al. 2004).

6.4. Dog (*Canis lupus familiaris*)

6.4.1. Species-specific information

Normal feeding is recommended once or twice daily in adult dogs; Water should be available *ad libitum* (TVT 2004, GV-SOLAS 2009).

In general, the combination of food and water deprivation and/or other factors, such as a change in the composition of the feed, increases the degree of severity (Home Office 2003).

6.4.2. Food deprivation

a) Mild

If only food is withdrawn and not in combination with other factors, the severity of exceeding a feeding interval once by up to 24 hours is classed as mild (Wu et al. 2002).

Weight loss in dogs should not exceed 10% over a week (Jones 1998) or 15% over a period of two weeks (NIH 2013).

b) Moderate

Based on the “mild“ and “severe” degrees of severity levels, the severity of a weight loss between more than 10% within one week and 15% within two weeks and a maximum of 20% is considered to be moderate.

The severity of exceeding the feeding interval once by more than 24 hours and up to 48 hours in dogs must also be considered moderate (UM-UCUCA 2014).

c) Severe

A weight loss of 20% or more compared with animals of the same age and sex not subject to food deprivation is considered severe.

Exceeding the feeding interval once by more than 48 hours must also be classed as severe.

6.4.3. Water deprivation

a) Mild

The severity of water deprivation for up to 12 hours in dogs is considered mild (van Vonderen et al. 2004, Fine et al. 2010).

b) Moderate

There is evidence to show that water deprivation for up to 24 hours leads to changes in the electrolyte and hormone balance and causes weight loss (Metzler et al. 1986, Reinhart et al. 2015). The severity here is classified as moderate.

a) Severe

Water deprivation that lasts longer than 24 hours is classed as severe (Zucker et al. 1982).

6.5. Sheep (*Ovis aries*)

6.5.1. Species-specific information

Sheep are ruminants and possess a relatively large stomach volume compared with non-ruminants (Mendel 2008). Water intake does not occur until after a time lag has passed following food intake (GV-SOLAS 2002) and is dependent on the composition of the feed, the climate, the housing conditions, the fleece and the performance (growth, lactation, recovery) of the animals (Mendel 2008, Spengler et al. 2015).

Animals that consume more concentrated feed (e.g. feed pellets, chopped hay) have a greater water requirement than animals whose feed ration has a higher fibre content (Aganga 1992).

Since food deprivation alone led to findings similar to those from sheep deprived of both food and water (Hecker 1964), food and water deprivation are addressed together.

6.5.2. Food and water deprivation

The rumen serves as liquid and energy reservoir, so food and water deprivation does not result in physiologically measurable dehydration until this reserve has been used up (Spengler et al. 2015). However, weight loss already occurs from the start of the deprivation (Hecker 1964).

As in most animal species, the restriction of water also leads to reduced food intake (Aganga 1992, Spengler et al. 2015).

After the withdrawal of food and water, the need for sheep to eat hay is greater than the need to drink water (Cockram et al. 1999). Water intake does not occur until after feeding (Kent 1997, Jackson et al. 1999, GV- SOLAS 2002).

Food and water deprivation lasting up to 48 hours in sheep did not lead to a change in plasma osmolarity or to an increase in the release of prolactin or cortisol (Parrot et al. 1996). On the other hand, however, the composition of the feed (including salt content, Meyer et al. 1955) before deprivation and housing conditions during deprivation (Aganga et al. 1986) had a decisive influence on weight loss during deprivation and the resulting severity of the deprivation for the sheep. The literature does not offer any standardized, comparable baseline data on this, so only the body weight of the animals and not the duration of the deprivation can serve as a basis for assessing the severity.

a) Mild

A weight loss of up to 5% is considered mild. For example, in a metabolic cage experiment with a prior adaptation period, water and food deprivation lasting 36 hours led to a weight loss of 5% (Meyer et al. 1955).

b) Moderate

A weight loss of up to 5% is considered mild. For example, in a metabolic cage experiment with a prior adaptation period, water and food deprivation lasting 36 hours led to a weight loss of 5% (Meyer et al. 1955).

c) Severe

Weight losses resulting from food and/or water deprivation that go beyond what is considered moderately severe are classed as severe.

6.6. Pig and mini-pig (*Sus scrofa*)

6.6.1. Species-specific information

When water and food are available *ad libitum*, pigs only consume food and drinking water irregularly. Marked variations occur in the daily ratio of food intake to water intake, especially in growing pigs (Stephens 1985; GV-SOLAS 1999). **The same principle applies here, that not every brief period of food or water deprivation can be regarded as essentially stressful.**

As with many other species, food intake and water intake in pigs are also closely linked with regard to timing as a rule. About 75% of the daily water intake occurs immediately before or together with food intake. As a result, physiological parameters, such as plasma osmolality or blood volume, are subject to only slight fluctuations with standard feed. The effects of water restriction or food restriction alone on such parameters are therefore often greater than those of food and water deprivation combined (Houpt and Yang 1995).

6.6.2. Food deprivation

a) Mild

The severity of food deprivation for 24 hours is mild (Fernandez et al. 1995; Tanaka et al. 2009).

b) Moderate

Since the withdrawal of food for up to 48 hours is accompanied by a weight loss of up to 15%, the severity of this food deprivation is considered moderate (Lallès and David 2011).

c) Severe

Based on the definition of moderate severity, the withdrawal of food for more than 48 hours is classed as severe.

6.6.3. Water deprivation

a) Mild

The severity of water deprivation for up to 12 hours in pigs can be regarded as mild (Stephens 1985).

b) Moderate

The severity of water deprivation for up to 24 hours in pigs is considered moderate (Knabe et al. 1986).

c) Severe

The withdrawal of food for more than 24 hours is accordingly classed as severe.

6.7. Non-human primates

6.7.1. Species-specific information

In non-human primates, as also in many other mammals, it is the case that - depending on the species - they can be adapted to arid habitats and hence naturally to situations where access to water is not always available. In such species, which include those frequently used in experiments such as rhesus monkeys (*Macaca mulatta*) and cynomolgus macaques (*M. fascicularis*), as well as more rarely used Barbary apes (*M. sylvanus*), water restrictions therefore have a less severe impact than they do in species from non-arid habitats (Lindburg 1977).

In behavioural studies in non-human primates given fluids as positive reinforcement, it is a good idea to include an adaptation phase in which the animals learn to meet their fluid requirements during the daily training sessions. From the outset, the amount of reward per test run must be adapted to the level of training at that moment, and it must be ensured that the animals receive rewards for as long as they want, i.e. until they permanently cease performing their respective task (DPZ 2007).

6.7.2. Food deprivation

For technical reasons, food rewards and thus food restriction play a minor role in experiments involving non-human primates. Reasons for this are both a high rate of test runs and also that a precise portioning and reward process matched to the correct behavioural response with pinpoint accuracy cannot as a rule be guaranteed with administration of feed portions. Therefore, recommendations for assessing the severity of food restrictions in non-human primates cannot be provided here.

6.7.3. Water deprivation

Phases of water restriction must be alternated with phases of *ad libitum* availability. Usually, the duration of water restriction and *ad libitum* availability is determined by the (weekly) working rhythm of the staff involved in the experiment. The following figures relate to the above-mentioned macaques.

a) Mild

Mild severity can be assumed when the following conditions are met:

- The average water intake does not fall below 20 ml/kg b.w. per day on five successive days during a five-day restriction phase or on three successive days during a 12-day restriction phase, and this is followed by at least one day of *ad libitum* availability (DPZ 2007, Yamada et al. 2010).
- For the reintroduction of water restriction following *ad libitum* availability, water is withdrawn for a maximum of 24 hours, which is counted towards the average of 20 ml/kg b.w. per day.
- A reduction of body weight caused by water restriction must not exceed 10% within a week and 15% over a period of two weeks in adult animals. Animals not yet fully grown must gain weight despite water restriction. To assess the severity of water restriction, colony-specific growth curves must be considered whenever possible,

and the weight gain must not show a sustained divergence from these curves (Prescott et al. 2010).

b) Moderate

Reduction of body weight between 15 and 20% over a period of two weeks.

In behavioural experiments, this degree of severity is general avoided, because the active participation of the animals that is usually absolutely essential to achieve the objective of the experiment is no longer guaranteed. Therefore, the literature provides no substantial information on duration and amounts of fluid during the restriction phases.

c) Severe

A reduction in body weight of more than 20% is classed as severe (see also comment for moderate severity).

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