Acclimatisation of rats before scientific experiments

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Independent Project • 30 credits
Swedish University of Agricultural Sciences, SLU
Faculty of Veterinary Medicine and Animal Science
Veterinary Medicine Programme

Uppsala 2023

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Acklimatisering av råttor inför försök

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Credits:	30 credits
Level:	Second cycle, A2E
Course title:	Independent Project in Veterinary Medicine
Course code:	EX1003
Programme/education:	Veterinary Medicine Programme
Course coordinating dept:	Department of Clinical Sciences
Place of publication:	Uppsala
Year of publication:	2023

Keywords: acclimatisation, acclimatisation period, rat, transportation, stress, corticosterone

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Abstract

The most common laboratory animals in Sweden today are mice, rats, and zebrafish. We sent out an anonymous digital survey to investigate if scientific facilities in Sweden acclimatise their rats after transportation, how long the acclimatisation time is, and how the acclimatisation is conducted. Studies have been made showing the effect transportation may have on rats. They have measured parameters such as corticosterone levels, heart rate, blood pressure, and behavioural changes. However, the recommendations given vary between 3 days and up to 2 weeks. Studies have also shown differences in acclimatisation times between females and males; where females will need longer for corticosterone levels to return to concentrations close to that before transportation.

Results from the conducted survey showed that a majority of research facilities acclimatise the rats after transport from the breeder; however, the acclimatisation times between facilities vary. Approximately 50% of the facilities acclimatise the rats for less than 7 days. Results from the survey also showed that a majority base the length of the acclimatisation time on experience/recommendations or that their main focus and base for the acclimatisation time was to reduce stress. Only a small percentage based the acclimatisation time on legislation or scientific studies. This may be due to the fact that neither European nor Swedish regulations on laboratory animals contain information and guidelines as to how long an appropriate acclimatisation time should be.

It's been proven that stress can have an effect on scientific results. This would constitute the importance of accurate acclimatisation before the experiment. More studies are needed on the subject in order to help and guide laboratory personnel to apply an accurate acclimatisation time catered to the individual group of rats used.

Keywords: acclimatisation, acclimatisation period, rat, transportation, stress, corticosterone

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Abbreviations

ACTH	Adrenocorticotropic Hormone
HPA-axis	Hypothalmic-Pituitary-Adrenal axis
LASA	Laboratory Animal Science Association
MAP	Mean Arterial Pressure
NACWO	Named Animal Care and Welfare Officers
NF-κB	Nuclear Factor Kappa B
SLU	Swedish University of Agricultural Sciences

1. Introduction

Each year in Sweden, thousands of animals are used in animal experiments. The Swedish Board of Agriculture reports the annual usage of laboratory animals in Sweden using the EU's definition of a laboratory animal, i.e., that have been subjected to some form of suffering equivalent to a pinprick, for scientific purposes. In 2018 they reported that approximately 6% of the total number of animals used were rats (Ljung *et al.* 2020). Article 5 of Appendix A of the European Convention (ETS no. 123) states that;

> "acclimatisation is needed to allow animals to recover from transport stress" and "The time required depends on several factors, such as the stress to which the animals have been subjected" (Council of Europe 2006 ETS No. 123, p. 13-14).

The description is both ambiguous and undefined as to how long this acclimatisation period needs to be. There is a need to investigate how long laboratory animals, and specifically rats in this study, are acclimatised after transportation and after rehousing within the facility. The primary purpose was to investigate how acclimatisation is conducted at research facilities in Sweden and what literature or other information is used to establish the length of acclimatisation. It was also of interest to investigate which aspects were important to the survey participants when choosing a breeder. The hypothesis was that the acclimatisation process would differ largely between Swedish facilities due to the lack of guidelines provided. This includes purchasing the animals, acclimatisation periods, and how the animals were acclimatised to their new environment. To investigate the hypothesis, a digital, anonymous survey was sent out through the Swedish 3R-center, to different research facilities in Sweden. The questions were designed to gather as much information about the acclimatisation process as possible and to better understand how acclimatisation is conducted nationally.

Studies have shown that rats are affected both when transferred externally and internally (Arts *et al.* 2014b). External transportation can cause a rise in corticosterone levels, weight loss, bradycardia, and loss of blood pressure amongst other things (Arts *et al.* 2012; Capdevila *et al.* 2007). The literature is however undecided on the length of acclimatisation time needed for the measured levels to stabilise. There is also evidence that there are differences between male and female rats, and that the time needed for acclimatisation should be adjusted due to the sex of the rats (Arts *et al.* 2014a; Figueiredo *et al.* 2002). There is a need for more studies to determine more clear guidelines for the length of time needed for acclimatisation. It would help research facilities to better understand why acclimatisation time is important and to get a more uniform acclimatisation process across research facilities in Sweden.

2. Literature review

2.1 Terms and definitions

Transportation of laboratory animals causes significant stress, which can greatly impact animal welfare (Swallow *et al.* 2005). Studies have shown that both external and internal transportation affects the animal both psychologically and physiologically, for example: behavioural changes and elevated corticosterone levels (Arts *et al.* 2014a; 2014b). In several of the studies, the authors usually recommend a period of time designed for the animals to acclimatise to their new environment after transportation. However, there are no clear-cut rules or statements for a minimum length of time. Article 5 of Appendix A of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS no. 123) states.

> "A period of acclimatisation is needed to allow animals to recover from transport stress, to become accustomed to a new environment and to husbandry and care practices." (Council of Europe 2006 ETS No. 123, p. 13).

It continues by saying;

> "The time required depends on several factors, such as the stress to which the animals have been subjected which in turn depends on several factors such as the duration of the transportation and the age of the animal and change of the social environment. It should also be taken into account that international transport may necessitate an extended period of acclimatisation due to disturbance of the diurnal rhythm of the animals" (Council of Europe 2006 ETS No. 123, p. 14).

Swedish laws and legislation contain little information regarding acclimatisation time. The Swedish statute regarding regulations on laboratory animals (SJVFS 2019:9) which regulates the usage of laboratory animals states that a Laboratory Veterinarian or other expert, in interaction with an ethologist, have a counselling role to ensure the animal's physical and psychological well-being. There should also be a written plan to evaluate the animal's physical and psychological well-being, as well as habituation and training programs which should be adapted to the animals and scientific experiments. Another section of the same legislation states that the ethical application used to approve the usage of laboratory animals in scientific experiments needs to include a description of how the animals will be kept and cared for before, during, and after the experiment (SJVFS 2019:9).

An important term used in the literature when describing physiological parameters stabilising, is allostasis (Arts *et al.* 2012).

McEwan (2002) discusses the term “allostasis” and describes it as *“maintaining stability through change”* (McEwan 2022, p. 921), while homeostasis is a system that operates in a narrow range and is essential for life. Suggesting allostasis as the term to be used to describe ´maintaining stability through change and promoting adaptation and coping´ (McEwan 2002).

2.2 The effects of stress

Abelson *et al.* (2005) state that;

> “Stress in laboratory animals is an obstructive circumstance in most experimental conditions, since stressors significantly alter the normal physiology and metabolism and, thereby, increase variation within and between individual animals. This makes stress a major source of experimental error “ (Abelson *et al.* 2005, p. 815).

As Abelson *et al*. (2005) describe, there is a risk of experimental error if the animals are stressed, since stress is known to affect the body in many ways such as the immune system, hormone secretion and behaviour (Fleshner *et al*. 1998; Swallow *et al.* 2005). Stress due to transport can therefore have an impact on both scientific validity but also animal welfare (Swallow *et al.* 2005). Studies in mice have also shown a decrease in reproduction after transatlantic shipping (Hayssen 1998).

The hypothalamic-pituitary-adrenal (HPA) axis is a neuroendocrine system. It consists of an endocrine pathway cascade that aims to regulate physiological functions such as: Immune response, central nervous system, and metabolism when the individual is affected by stressors (Sheng *et al.* 2021).

When exposed to a threat or a stressor, hormones will be released from the adrenal gland affecting physiological mechanisms such as: Vasoconstriction, and energy preservation, to name but a few (Papadimitriou *et al.* 2009).

Glucocorticoids are steroid hormones produced in the cortex of the adrenal gland (Loeb & Quimby 1999) and affect the HPA-axis by acting as a negative feedback system (Zachary 2017). Glucocorticosteroids are also regulated by another hormone called adrenocorticotropic hormone (ACTH) which is produced in the pituitary gland (Papadimitriou *et al.* 2009). The negative feedback should act as an inhibitor of glucocorticosteroids and repress the effects of the HPA-axis (Franco *et al.* 2016), however, during stress, the pituitary function is disrupted by nuclear factor kappa B (NF-**κ**B) activation, thus exaggerated ACTH-secretion affecting the secretion of glucocorticosteroids instead of it being inhibited (Papadimitriou *et al.* 2009). One important glucocorticoid is corticosterone which is predominantly produced in rodents (Loeb & Quimby 1999).

The effect of adrenal steroids on the cell is to increase the synthesis of cell alteration proteins as well as promote the reduction of protein synthesis in organs and tissues of the immune system. The effects of glucocorticoids are several;

including enhancement of gluconeogenesis, as well as increased storage of glycogen (Miller *et al.* 2002). In an emergency situation, an individual's metabolism increases, and glucose provides the much-needed energy for the stressful situation. It also possesses anti-inflammatory effects such as the immobilisation of monocytes and neutrophils to an inflamed location, as well as a decreased immune response with a lower number of monocytes, B lymphocytes, and T lymphocytes (Loeb & Quimby 1999*).*

Stress can both be acute and chronic, and the immune system can be affected by both. Acute stress can cause the suppression of the adaptive immune system, and studies in rats have shown that acute stress can lower the production of T cells, specifically a Th1-like subset (Fleshner *et al.* 1995). Acute stress can also increase immune responses, more specifically the innate immune response (Deak *et al.* 1999; Fleshner *et al.* 1998). Chronic stress has also been shown to decrease the response of the adaptive immune system in mice (Tournier *et al.* 2001). When stressed, rats' blood corticosterone levels will rise and can therefore serve as a good biomarker for stress (Abelson *et al.* 2005).

It is important to note that corticosterone levels display a circadian rhythm in rats and many other species (Loeb & Quimby 1999). It’s been shown that most hormones are not secreted at a constant rate, but rather in a cyclic or rhythmic manner. The rhythm of secretion within the animals can be affected by the environment, an example of an environmental cue is the light and dark cycle. A circadian secretory rhythm is a term which describes a pattern of secretion where hormone concentration will be different at certain times of the day. Hormone concentrations will be at its lowest during a specific part of the day and then peak at high concentrations at other times. The circadian rhythm can therefore affect research results if the blood samples are collected at different times. In rats, corticosterone and ACTH levels are at their highest at the beginning of the dark phase and at their lowest at the beginning of the light phase. There are also differences in stress hormones between the sexes where female rats experience higher levels of corticosterone than male rats. Corticosterone and ACTH levels are highest in late proestrus but at their lowest in estrus. Studies have shown that there might be a correlation between high corticosteroid blood levels for a longer period of time and the estrus cycle (Figueiredo *et al.* 2002). One study used male and female rats to measure corticosterone levels after a stressor. The female rats were either in proestrus, estrus or diestrus. By exposing the rats (both male and female) to a stressor, and then sacrificing the rats at different lengths of time after the stressor, the authors could see that females in proestrus and estrus had higher levels of corticosterone levels for a longer period of time than their male counterparts.

Morton *et al.* (1985) describes signs of distress and discomfort in experimental animals. The authors also describe a hypothesis for the assessment and characterisation of several signs seen in laboratory animals which can be linked to pain, distress,

or discomfort. Such signs are: Alteration of heart rate, loss of body weight, poor growth, and rapid or otherwise abnormal breathing among several others. Signs of distress can also be shown in the behaviour of the animals. Such behavioural changes can be related to posture changes or changes in temperament such as being more docile or aggressive.

Behaviour in rats can also differ depending on the sex. Evidence suggests that female behaviour is more easily disrupted than that of males due to stress (Dalla *et al.* 2005).

Stress can not only affect bodily functions (Abelson *et al.* 2005; Deak *et al.* 1999; Fleshner *et al.* 1998; Loeb & Quimby 1999; Morton *et al.* 1985) but can also have an effect on the outcome of research results (Shima *et al.* 2009). A study made by Shima *et al.* (2009) showed that biological mechanisms and physiological effects can be affected after stress and this needs to be taken into consideration when establishing the acclimatisation time.

One such study investigated the known anorectic effect of gastrointestinal hormone peptide YY_{3-36} in rats and humans and how that hormone might be altered due to stress (Abbott *et al.* 2006). The rats were injected with the peptide hormone and then subjected to mild stressors. The results showed a decrease in the peptide hormone effect compared to their controls. Another study done on mice with a similar result suggests that the decrease in the efficiency of the peptide hormones could be a result of stress (Halatchev *et al.* 2004).

Another important aspect to include is the environmental changes rats will experience after moving from the breeder to the laboratory (Arts 2016). The transfer between the breeder and laboratory is often a process which includes several elements which can be a source of stress. At the breeder, the rats are normally kept in standardised conditions with a suitable and regulated temperature, humidity, and enrichments. The enrichment in the cages can also help them regulate their own temperature to a suitable level.

One such breeder and supplier of laboratory rats, Taconic, describes the housing at their facility (Taconic 2022). The conditions the animals are kept in at their breeding facility include: Housing rats in groups and providing enrichment to promote normal behaviour for the species and well-being. Also controlling the temperature, humidity, noise levels, and light-dark cycles to reduce stress and provide good living conditions.

Neither a regulated temperature nor enrichment is normally present during the transport which can cause temperatures that are either too high or too low for the rats during transfer (Arts 2016). This may in turn cause difficulties when the rats need to adapt to their new environment at the laboratory after the transfer from the breeder.

2.3 Acclimatisation and recommendations

Even though there is a limited number of studies made on the effects of transport in laboratory animals, some studies have been done on rats and the effect of transport and recommendations for acclimatisation periods. One such study was made to measure the length of time rats needed for their physiological parameters to return to a steady state after ground transportation (Capdevila *et al.* 2007). Ten rats were fitted with radiotelemetry trackers and transported intracontinental with no light-dark shift, in a vehicle for 5 hours. The rats exhibited both lower body weight and bradycardia after transport. Similar results have also been shown in guinea pigs after ground transportation (Stemkens-Sevens *et al.* 2009). Capdevila *et al.* (2007) concluded that 3 days were needed for the rats to return to normal parameters in heart rate, body weight, and activity level, after transport. Another study by van Ruvien *et al.* (1998) investigated nutritional parameters after intra-continental transportation and came to similar conclusions regarding acclimatisation time. This study transported the rats by aeroplane as well as ground transport to and from the airport. The authors concluded that approximately a 3-day acclimatisation period was sufficient. Notably, the measured temperature and humidity changed drastically during transport. The humidity ranged from 21.0-100% and the temperature range was between 17.0-28.5℃ (van Ruiven *et al.* 1998).

Arts *et al.* (2012) used 108 male rats in their study to observe the acclimatisation time needed for the measured parameters to return to allostasis. In this study, researchers measured the rats' corticosterone levels, body weight, and behavioural observations as well as heart rate, blood pressure (MAP), and activity rate measured by radiotelemetry. The study used 108 male rats, 27 of the rats were transported externally. The 27 rats were divided into nine cages, three rats in each cage, and one rat in each cage had been fitted with a radiotelemetry device. One of the control groups consisted of 27 rats, three rats in each cage, and four rats had functioning radiotelemetry devices. These rats were not transported but bodyweight, heart rate, MAP, corticosterone levels, and behavioural observations were made. The other two control groups consisted of 27 rats respectively, three rats in each cage, none of these rats had a radiotelemetry device fitted. One of the control groups without radiotelemetry devices, were packed but not transported. Body weight and corticosterone levels were measured, but no other physiological parameters were measured. The other control group without radiotelemetry devices, were not packed or transported. Bodyweight, behavioural observations, and corticosterone levels were measured. Behavioural observations were made which included, but were not limited to: Social interaction and grooming behaviour. The results showed that the transport (via vehicle) from the breeder to the research facility affected the rats greatly. The rats exhibited both reduced MAP and bradycardia which took approximately four days to stabilise. However, neither heart rate nor MAP fully returned to baseline levels before transport, illustrating allostasis rather than

homeostasis. The transported animals also showed significantly higher corticosterone than that of the control groups. One group was packed but never transported, this group also displayed higher corticosterone levels than the other controls. The author mention that corticosterone levels were elevated in both groups receiving a transmitter. The author theorise that the increased levels of corticosterone could partially be due to a postsurgical effect and that the recovery time after surgery may not have been long enough. The authors go on to say that the recovery period after surgery could have been extended. Increased grooming was also an observed behaviour in the transported animals. This behaviour returned to baseline after 1 week. The authors' recommendations for acclimatisation time are not straightforward and conclusions and recommendations cannot be established for all rats since the study only included male rats. Their definition of acclimatisation was *"a period of 3 successive days in which parameters are on a stable level"* (Arts *et al.* 2012, p. 13). Acclimatisation based on heart rate and MAP would be 1 week while it took 3 weeks for corticosterone levels to stabilise (Arts *et al.* 2012).

Animals sometimes must travel far to reach their new destination. Shim *et al.* (2009) transported 20 rats from New York, USA, to their research facility in Korea. Two groups were used for transportation. The third group, which was the control, were of the same strain, bred at the Korean facility from animals bought from the breeder in the USA one year earlier. The main focus was to investigate corticosterone levels after transport. Both male and female rats in the transported groups demonstrated a significant increase in corticosterone levels compared to the controls. A one-week acclimatisation period resulted in a decrease in blood corticosterone levels in both sexes compared to the control group. Results from the study also showed elevated levels of HSP70 and GRP78 which are stress proteins and good biomarkers for stress. The researchers saw elevated levels of the proteins in the heart, brain, kidneys, lungs, liver, and muscles after transport. After the acclimatisation period, the levels of HSP70 and GRP78 were mostly restored to normal levels compared to the control groups, however, in some tissues such as the kidney, heart and muscles, the protein levels were not fully restored to normal levels after one week.

Some studies indicate a need for different acclimatisation times depending on the sex of the animal (Arts *et al.* 2014a; 2014b). Elevated corticosterone levels for a longer period of time after stressors have been correlated with females in proestrus and estrus (Figueiredo *et al*. 2002).

Arts *et al.* (2014a) studied differences in animal welfare between male and female rats after transportation. The authors saw a difference in behaviour. Female rats expressed a decrease in social interaction but an increase in self-grooming. This behaviour stabilised 3 weeks after transportation. In males, however, there were no significant changes in behaviour after transport. There were noteworthy differences in the corticosterone levels between the sexes where female corticosterone levels

were higher than the males. These differences were not only limited to the transported animals (Arts *et al.* 2014a) but also in line with literature in general (Kitay 1961). Acclimatisation recommendations were 1 week for males and 2 weeks for females (Arts *et al.* 2014a).

A similar study looked at the difference between sexes as well as the external and internal transfer of animals (Arts *et al.* 2014b). There was a significant difference in corticosterone levels between males and females, both when externally and internally transferred. The corticosterone levels in females were increased several days after transfer compared to their male counterparts. Transfer also affected heart rate differently in the sexes, where the transferred females had an increased heart rate and males experienced bradycardia compared to their control groups and concluded that females might show other different physiological parameters in response to stress compared to males. An acclimatisation time of 2 weeks when transferring animals externally was recommended. They also took the previous study Arts *et al.* (2014a), into consideration when recommending this time period as well as the results from this study, mentioning that the commonly recommended 1-week acclimatisation would not be sufficient according to their results. Conclusions that experimental procedures should be postponed for 8 days in males and at least 2 weeks for females after being transferred externally were also recommended (Arts *et al.* 2014b). They also recommended that experimental procedures should be postponed for 2 days when transferring animals internally.

It is important to understand the effect light might have on rats when transporting and housing the animals. It's been shown that intense light conditions disrupt and suppress social and playful behaviour in juvenile rats (Vanderschuren 1995). The circadian rhythm has been shown to have an impact on blood pressure and heart rate (van den Buuse 1999; Zhang *et al.* 2000).

Zhang *et al.* (2000) implanted telemetry transducers in 14 Wistar rats and recorded activity, blood pressure, and heart rate during a 12/12-hour light and dark period over five weeks. The light and dark period was then shifted for 6 following weeks by shortening the light period by 4 hours, dividing the light-dark period by 8/16-hours respectively. This was done by either extending the dark period by 4 hours at the beginning of the night (experiment 1) or by extending it by 2 hours at the beginning of the night and decreasing it by 2 hours at the end of the night (experiment 2). The light-dark period was then reverted back to a 12/12-hour cycle. Data collected before the prolonged dark period showed a distinct 24-hour circadian rhythm in locomotion, systolic, and diastolic blood pressure, and heart rate. In both experiments, there was a significant change in the acrophase of heart rate, locomotion, and blood pressure. After the return to a 12/12-hour light-dark cycle, it took between 1-3 weeks before the acrophase of the measured physical parameters returned to normal levels.

On a similar line, one study measured blood pressure, heart rate and activity levels with surgically added telemetry implants in rats (van den Buuse 1999). Data was first recorded during a 12/12-hour light-dark cycle. The light phase was then extended by 6 hours making the 24-hour period 18 hours of light and 6 hours of dark. After 7 days the light-dark phase was reversed into 6 hours of light and 18 hours of dark period for 7 days before reverting the light-dark cycle back to 12/12-hours. Each time the rats were allowed to adapt to the new cycle for 7 days. The 6-hour light-dark shift caused an immediate change in heart rate, blood pressure and activity levels. In both shifts, it took approximately 4 days for the blood pressure to stabilise to "normal values", and 5 days before the heart rate stabilised. The acrophase also showed a significant change during the shift, taking approximately 3-4 days to stabilise, similar time was shown for activity levels to normalise. The order and time frame in which the light and dark change was made during van den Buuse (1999) experiment, has been summarised to increase comprehensibility.

1. 12 hours of light period and 12 hours of dark period for 7 consecutive days.
2. Light period is extended by 6 hours = 18 hours of light period and 6 hours of dark period for 7 consecutive days.
3. The light and dark period is reversed = 6 hours of light period and 18 hours of dark period for 7 consecutive days.
4. Light and dark period is reverted back to a 12/12-hour schedule = 12 hour light period and 12 hour dark period.
5. Result: Both light-dark shifts described in point 2 and 3 resulted in changes in blood pressure, heart rate and activity levels. It took 4-5 days to stabilise and 3-4 days for the acrophase to stabilise.

3. Material and method

3.1 Survey model

An anonymous survey (Appendix 1) was developed to investigate how the acclimatisation of laboratory animals is managed before animal are used in experiments at research facilities in Sweden. The purpose of the questions was to gather information about the three most common animal species used in scientific research in Sweden (Jordbruksverket 2020 Table 1), i.e., mice, rats and zebrafish. There was one survey for each species (mice, rats and zebrafish) but containing the same questions. All questions were available in both Swedish and English. The survey questions were written by the author of this paper and by the author of "Acclimatisation of mice before animal experiments" (Karlsson 2023). The questions were then reviewed by the supervisors and a representative from the Swedish 3R-centre, which all hold expertise in the usage of animals in experiments and the processes and work that it includes.

The survey questions were divided into four focus sections with a descriptive heading to indicate what information would be relevant to gain from each section. The focus sections were:

1. Basic information. This category gathered basic information about the participant and if acclimatisation of laboratory animals is a normal practice at their research facility.

2. Planning. To gain knowledge as to what sources are normally used when planning the acclimatisation.

3. Purchase of animals. How far do the animals have to travel, what is important when choosing a breeder, and is animal welfare taken into consideration when selecting a breeder?

4. Practical implementation when acclimating rats. Who oversees the acclimatisation, how is it done, and is there a control system in place?

The survey consisted of 29 questions and was a mixture of statements where the participant could answer Yes/No/Do not know (with an option for further comments on the question), multiple choice questions, or questions where the participant was asked to describe how specific parts or scenarios of the acclimatisation process were executed at their research facility.

Every question was available to every participant and based on the answers given they could skip questions that were not applicable. The survey was therefore adapted based on the participants' answers. For example, if a participant did not

practise acclimatisation of their laboratory animals or did not know if acclimatisation of laboratory animals was conducted at their facility, questions regarding how acclimatisation was conducted at their facility were not presented for the participants to answer.

The questions were entered into a program regularly used by the Swedish 3R-center, APSIS Pro, created by Apsis International AB and part of the organisation Efficy Group. It permits the editor to choose if the participants' answers should be anonymous, this application was added to ensure complete anonymity by the participants taking this survey.

The survey included an introduction explaining the purpose of the survey, that it was sent by the Swedish 3R-centre in collaboration with SLU, whom the survey was primarily targeted to and approximately how long the survey would take to answer. Before the survey was officially sent out, it had been answered by two volunteers to test how long it would take to complete the survey. All answers given in the survey were extracted into a spreadsheet which also included the time and date the survey was taken, which language and the participants' answers and comments.

The survey includes questions and an introduction letter which can be viewed in Appendix 1. The survey in Appendix 1 is in the same format as when it was sent out digitally to the participants. All questions marked with a purple colour were obligatory to answer to be able to proceed with the survey.

3.2 Survey distribution and target population

The Swedish 3R-center, a part of the Swedish Board of Agriculture, distributed the survey digitally. The survey was sent out by email where the recipient/participant was provided with three different links, one for each species. The participant was asked to answer the survey for the species with which they worked. The survey could be taken more than once depending on which laboratory animal or animals the participant worked with. The contact information was taken from a database, using criteria to reach the correct target population with the survey. This included anyone registered with the Swedish 3R-center as a member of an Animal Welfare Body in Sweden, a Laboratory Animal Veterinarian, or an Animal Technician. The target population also included researchers that had applied for ethical approval between the years 2018-2022. Anyone who previously asked the Swedish 3R-center to receive information about their work was also included as a possible recipient. The survey was also able to be shared by anyone receiving the email. The true number of recipients of the email is there for unknown. The email list was requested on 30/08/2022, the survey was sent out on 30/08/2022, a reminder was sent out on 13/09/2022, and the last day to participate was 20/09/2022.

3.3 Processing data

When the survey was closed, the data was extracted by the Swedish 3R-center into an Excel spreadsheet. The spreadsheet was uploaded to a Google Drive and the program used to manage the data was Google sheets. The data was then sorted to only show answers from completed surveys. Each question was then sorted one at a time and each answer was counted by marking each same answer. The summary of all marked answers was then displayed at the bottom right corner of the document and noted. The answers from each question were written down and sorted into groups according to job title, who answered the question (Researcher, Laboratory Assistant, Animal Health Professional, Laboratory Animal Veterinarian, Named Animal Care and Welfare Officer (NACWO), or Animal Facility Manager) and answer i.e. Yes/No/Do not know, multiple choice or free text. The percentage was calculated by the following formula; =(data point)/(total value)´. Each answer given was read by the author of this dissertation and some answers can be seen in the results section. Some questions that the participants answered in free text have been grouped together based on the answers given and displayed in the results section. How this selection and grouping have been made is described in the results section.

4. Results

A total of 38 participants completed the survey and answered every question. 50% of the participants who took the survey were researchers, and the remaining 50% marked themselves as working as either a Laboratory Assistant, Animal Health Professional, Laboratory Animal Veterinarian, Named Animal Care and Welfare Officer (NACWO), or Animal Facility Manager, referred to as Other in Figures 2 and 3. Figure 1 shows the distribution among the participants. The full survey including questions, answers, and comments from the participants, can be viewed in Appendix 2.

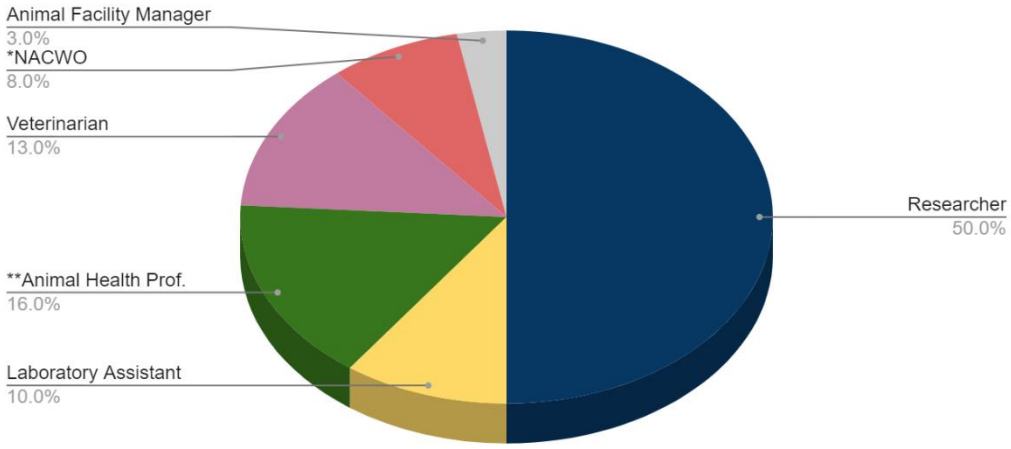


*Figure 1. Chart showing the distribution of survey participants and their job titles at their research facility. *NACWO = Named Animal Care and Welfare Officer, **Animal health prof. = Animal Health Professional.*

The participants were asked if they acclimatise the rats at their research facility. Out of the 38 answers, 36 participants stated that they acclimatise rats before animal experiments. One participant stated that no acclimatisation takes place and one participant stated that they did not know if any acclimatisation took place at their research facility. The participants who answered ´No´ or ´Do not know´ did not have to answer any more questions regarding acclimatisation at their research facility. The participant who answered ´No´ commented that they did not acclimatise the rats to any special conditions. The participant who answered ´Do not know´ also added a comment writing ´Both Yes and No´. Participants answering ´Yes´ were directed to more questions regarding the acclimatisation of rats at their research facility. One participant who answered that they do acclimatise

rats before experiments also added a comment explaining that acclimatisation is not always conducted and that it depends on the study. The participant explained that if the rats were in a pharmacokinetics study (PK-study) they were not acclimatised prior to the experiment. Results can be seen in figure 2.

Job title	Yes	No	Do not know
Researcher	18	1	0
Other	18	0	1

Figure 2. Answers given by the participants to the question ´Are rats acclimatised at your research facility?´. The group ´Other´ includes Laboratory Assistant, Animal Health Professional, Laboratory Animal Veterinarian, Named Animal Care and Welfare Officer (NACWO), or Animal Facility Manager.

The participants who answered ´Yes´ when asked if the rats were acclimatised before experiments were given follow-up questions regarding the acclimatisation plan. One question was aimed to investigate if research facilities have a written acclimatisation manual which describes how the rats should be acclimatised when arriving at the research facility. A total of 50% of all researchers did not know if they had a written manual for acclimatisation and 17% answered that they did not have a written manual at all. Participants named as “Other”, answered that 61% had a written acclimatisation manual while the rest did not have a manual or did not know if they had a manual for acclimatisation. The results can be seen in figure 3.

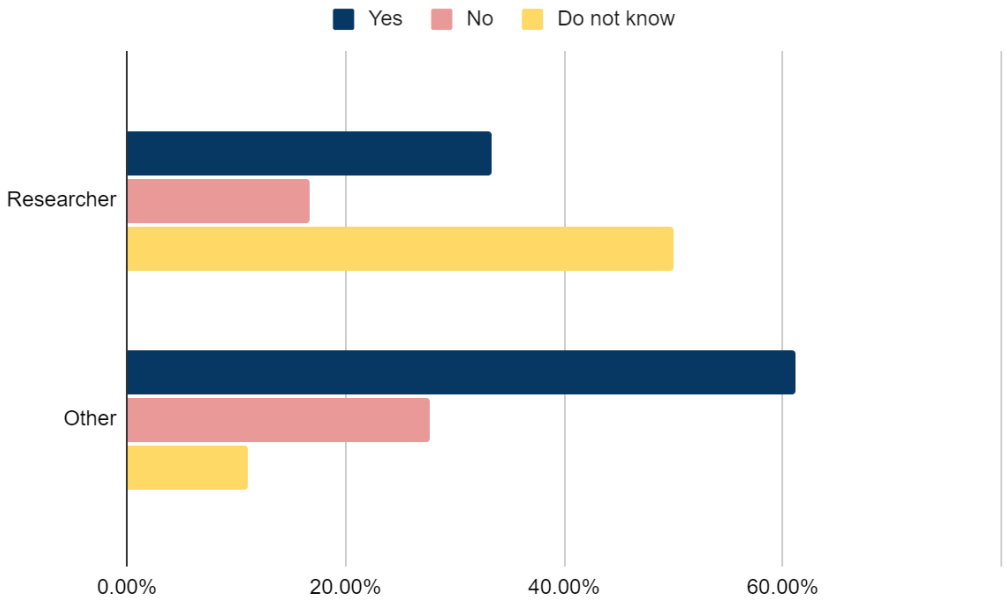


Figure 3. ´Question: Does your research facility currently have a written manual describing how the acclimatisation of rats should be conducted prior to trials?´. The group ´Other´ includes Laboratory Assistant, Animal Health Professional, Laboratory Animal Veterinarian, Named Animal Care and Welfare Officer (NACWO), or Animal Facility Manager.

The participants were asked if the rats get time to acclimatise, and for how long when they arrive at the research facility. The participants had to answer in free text. For the results to be more easily presented in a graph, the participants' answers have been divided into three different groups as follows. The rats get acclimatised for <7 days, the rats get acclimatised for 7 days and, the rats get acclimatised for >7 days. Some answers from participants stated a time interval for the length of the acclimatisation, citing that the acclimatisation was 3-5 days, 1-2 weeks etc. The answers have been grouped into <7 days, 7 days or >7 days based on the least amount of time stated in the answer. For example, if a participant would state that the acclimatisation time is 1-2 weeks, the shortest acclimatisation time that may occur is 7 days. If the participant wrote that the acclimatisation time is 5-7 days, the participant's answer would therefore be grouped in the category ´<7 days´. The results can be seen in figure 4. Some participants added comments to this question explaining that depending on the experiment or certain circumstances, the acclimatisation time can be shortened to as little as 24 hours or sometimes the rats will not be acclimatised at all. Investigations regarding acclimatisation time also included questions regarding if the acclimatisation would change depending on the purpose of the research. A total of 33% answered ´Yes´, that the acclimatisation would change, 53% answered ´No´ and 14% answered ´Do not know´. Anyone answering ´Yes´ to the question was later asked to describe how the acclimatisation would change. Several participants described that the acclimatisation times may be shorter or longer depending on the purpose of the research. Others described that the way the rats were handled might be changed depending on the research purpose. The results can be seen in figure 5. All but one comment made by the participants have been translated from Swedish to English by the author.

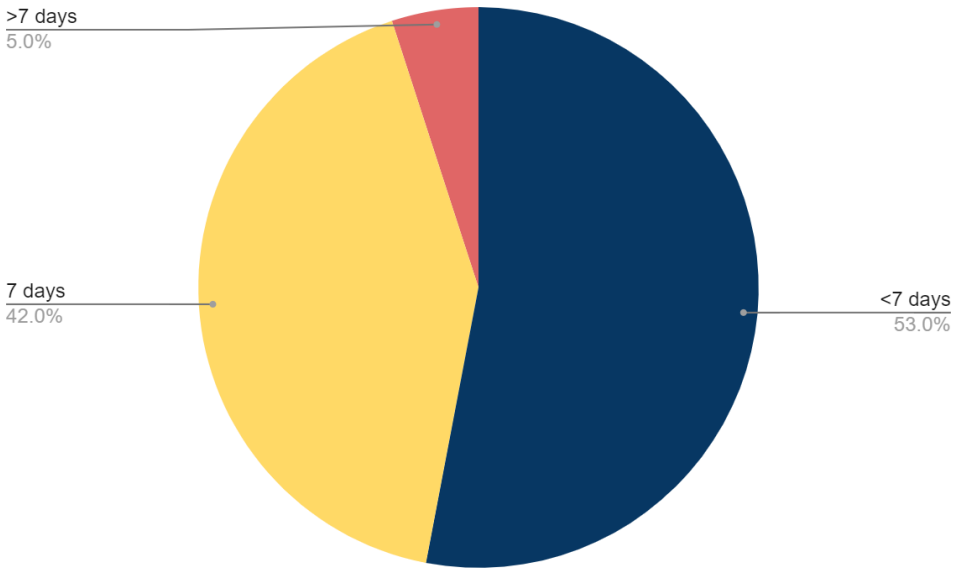


Figure 4. `Question: Are rats acclimatised when first arriving at your facility? If Yes, please note for how long`.

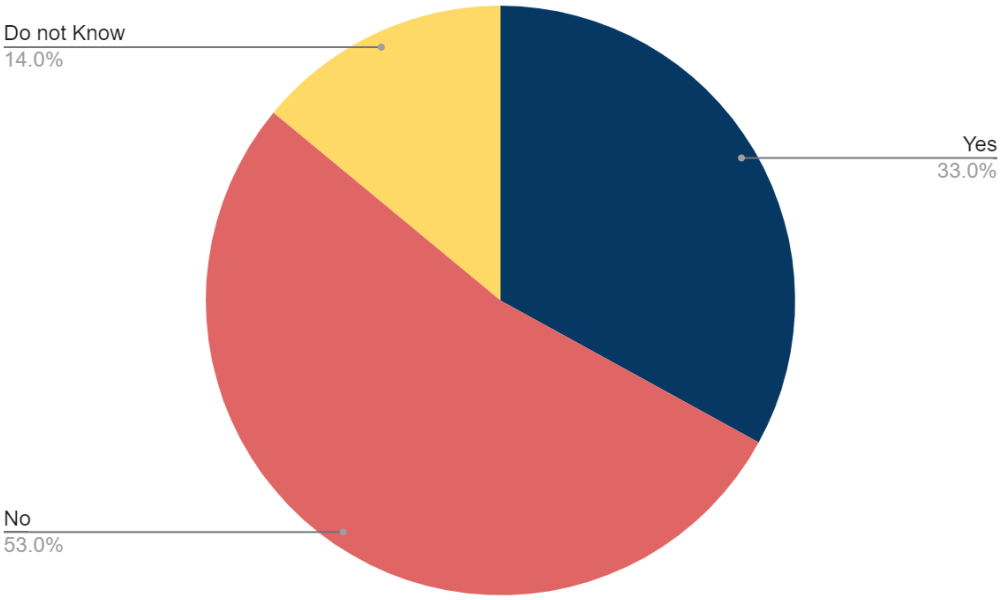


Figure 5. `Question: Does the plan for acclimatisation of rats change depending on the purpose of the research, for example, if the purpose of the research is a behavioural study or a terminal trial?´

Below are comments from anonymous participants. A full account of all answers can be viewed in Appendix 2. The question asked was ´Please describe the changes that are made to the acclimatisation process based on the purpose of the research.´

Before behavioural studies, we handle the animals for several days beforehand, in comparison to any terminal studies.

Behavioural studies have a need for calm animals while terminal studies and surgery do not have the same needs.

For short experiments, we only house the rats for a minimum of 5 days. During chronic experiments, the rats will be weighed and handled for at least 7 days.

It may take up to 8 weeks to accomplish a baseline standard. Especially with young animals. But this depends on the research topic, e.g. behaviour, endocrine mechanisms and biorhythmic.

Before behavioural studies, greater habituation can be conducted with the researcher as it may be good for the project.

Terminal experiments 5 days, chronic experiments 7 days. In some cases, there is no acclimatisation at all (rare).

For terminal studies, the animals may be taken earlier, otherwise there are 5 acclimatisation days as a standard.

The survey also included questions regarding acclimatisation to other changes in living situations for the rats. These questions included if the rats get acclimatised after re-grouping (Figure 6), when moved within the research facility (Figure 7) and if there is an acclimatisation period when there is a change in the light-dark period (Figure 8). When asked if rats are re-grouped at the research facility, one participant answered that they acclimatise the rats if they are re-grouped but they were not sure if others at their facility do the same. Another participant answered that the rats get acclimatised for 2-4 days. These two answers were interpreted as ´Yes´ i.e., that they do acclimatise the rats when regrouped. Also, when asked if the rats get acclimatised after an internal transfer within the facility, three participants answered the question by writing the length of time the rats were acclimatised for when moved within the research facility. The acclimatisation times given were 1h, 3 days and 5 days. These answers were interpreted as ´Yes´ i.e., that they do acclimatise the rats when moved within the research facility.

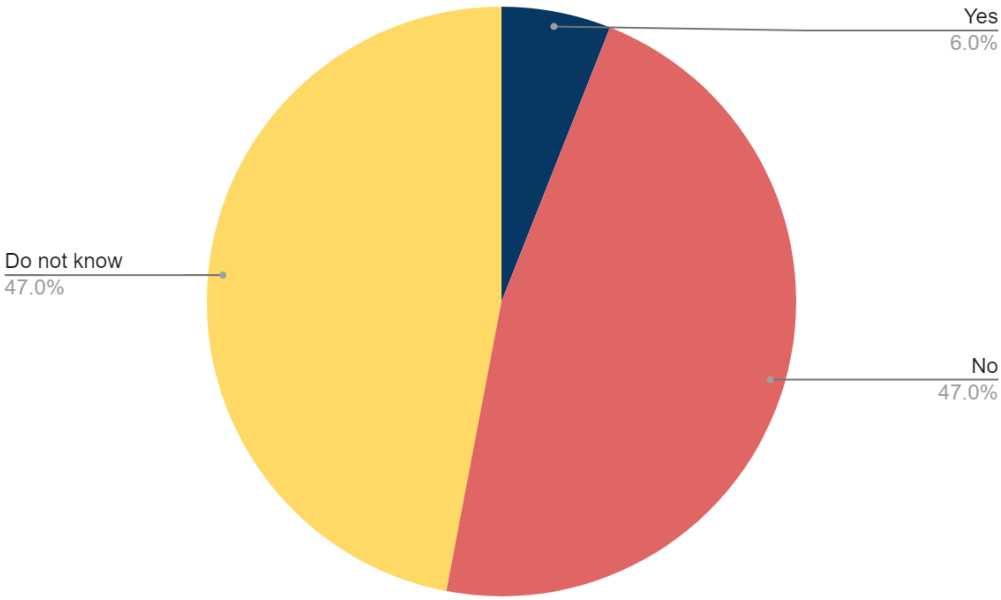


Figure 6. `Question: Are rats acclimatised when they are re-grouped at your facility?`. The majority of participants answered ´No´ or ´Do not know´.

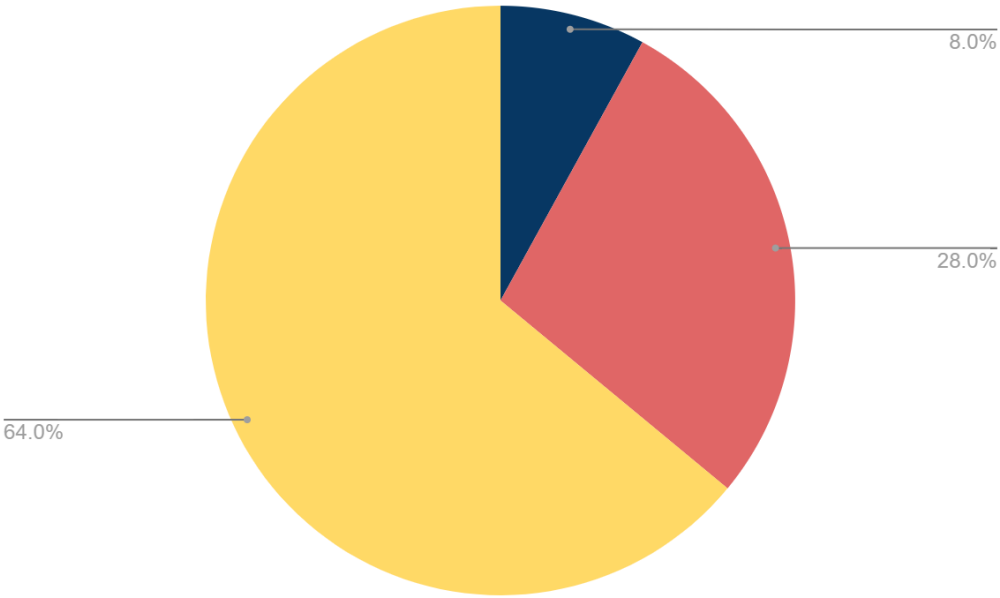


Figure 7. ´Question: Are rats acclimatised when they are moved to a new location within your facility?´.

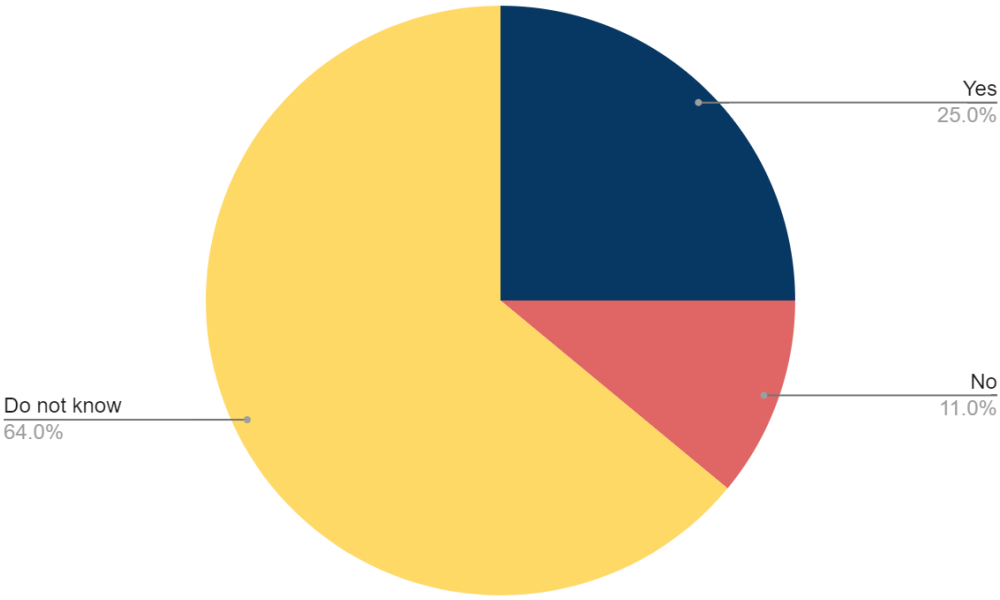
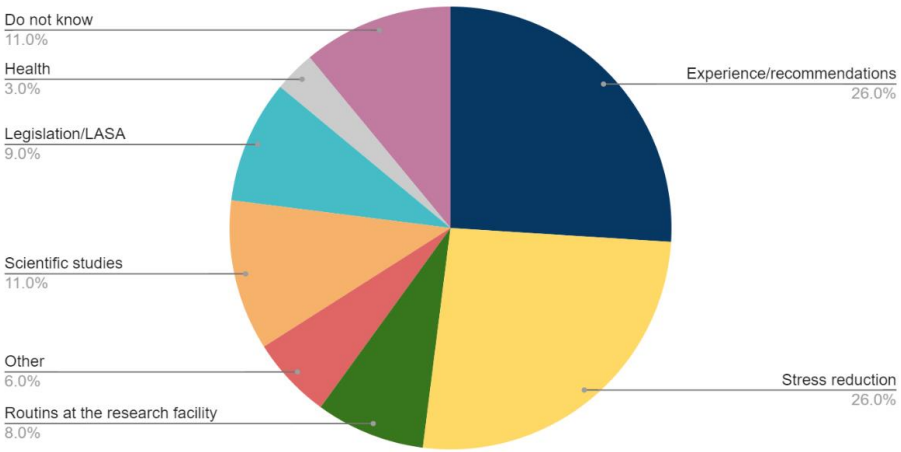


Figure 8. ´Question: Are rats acclimatised when the circadian rhythm is changed at your research facility? ´.

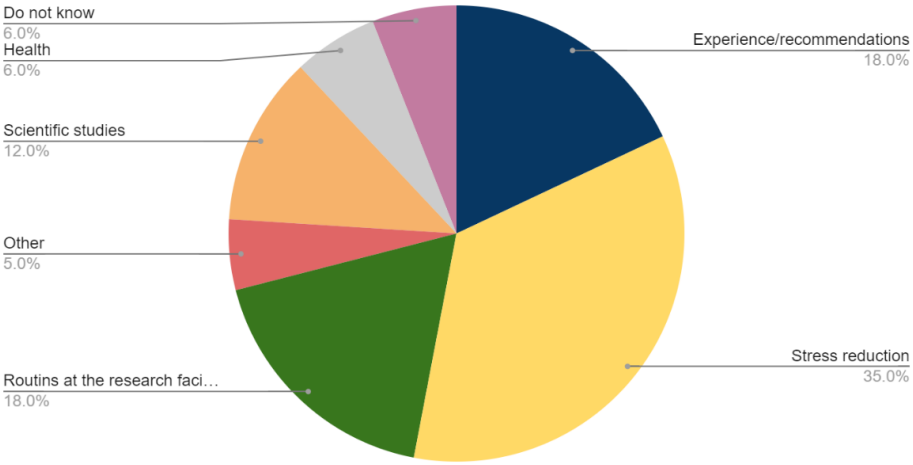
If a research facility acclimatises the animals before experiments, it was of interest to know what they based the acclimatisation on. In the survey, the participants were asked to shortly describe, in free text, the main reasons used when deciding the acclimatisation time. The answers have been divided into different groups based on

the main focus of the acclimatisation and/or what the participant specifically has stated as the source they used to establish the acclimatisation time. This means that the answer needs to contain a keyword such as Experience, Legislation, Scientific data etc to be grouped as such. If the answer did not contain a keyword but expresses, for example, that the main focus was to have ´calm´ rats or that the rats ´should not be scared, it has been interpreted that the main focus is ´Stress-reduction´ and has been counted as such. The groups include:

1. Experience/recommendations. If the primary source for the acclimatisation time is based on experience or recommendations.
2. Scientific studies. If the primary source for the acclimatisation time is based on scientific studies.
3. Legislation/LASA (Laboratory Animal Science Association). If the primary source for the acclimatisation time is based on legislation and/or LASA guidelines.
4. Health. If the main focus for acclimatisation is to make sure that the animals are healthy.
5. Stress reduction. If the main focus of acclimatisation is to reduce stress.
6. Routines at the facility. The length of the acclimatisation time is according to the research facilities' guidelines and routines
7. Do not know.
8. Other. If the primary source or main focus for acclimatisation is non of the above.



A.



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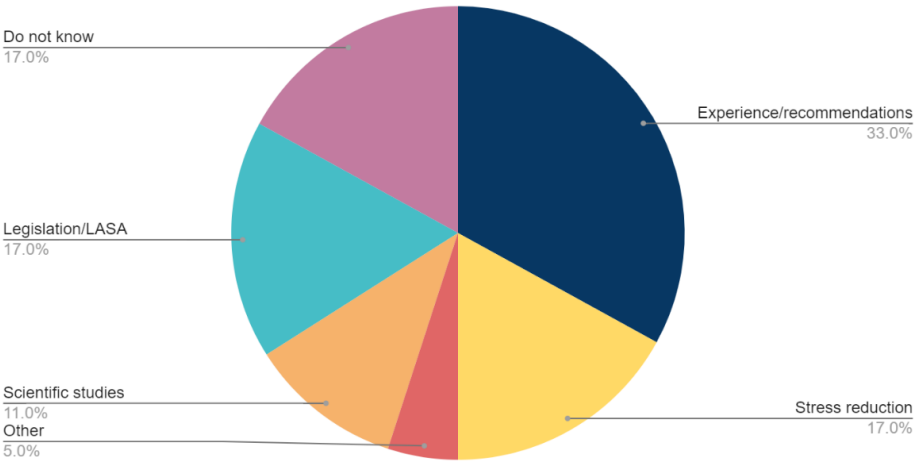

C.

Figure 9. ´Question: What are the main reasons used when deciding the length of acclimatisation of rats? ´ Answer distribution in (A) all respondents, (B) the category ´Researcher´ and (C) participants in the category "Other". The group ´Other´ includes Laboratory Assistant, Animal Health Professional, Laboratory Animal Veterinarian, Named Animal Care and Welfare Officer (NACWO), or Animal Facility Manager.

Figure 9A-C show the distribution of answers in regards to what the participants based their acclimatisation time on. In figures 9B and C, the answers have been broken out into categories where figure 9B shows the answer distribution from researchers only and in figure 9C are the answers from the category “Other”. In figure 9A the division between acclimatisation time based on Experience/ recommenddations and Stress reduction are the same, approximately 26% each. However, in figure 9B a higher percentage of researchers have answered that the acclimatisation time is based on stress reduction rather than experience/ recommendations, while the opposite is true for the group “Other” (Figure 9C). Approximately the same percentages in both categories have answered that the acclimatisation time is based on scientific studies. No one in the category ´Researcher´ answered that the acclimatisation time was based on legislation or LASA guidelines. In the category `Other`, no one answered that the acclimatisation time was based on ´Routines at the research facility or ´Health´.

A section of the survey focused on gathering information regarding transportation time and important factors when choosing animal breeders. The survey showed that the majority of all rats used in scientific experiments came from a breeder (Figure 10) and that 89% of all animals were transported from an EU or EES-country that is not Sweden, while 3% of transported rats came from a Swedish breeder (Figure 11).

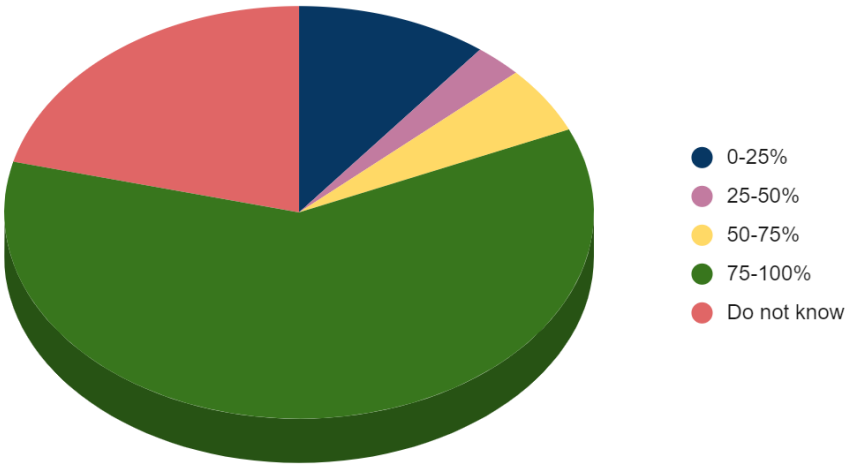

Figure 10. ´Question: Approximately what percentage of the rats that are used at your facility have been purchased from a supplier? ´

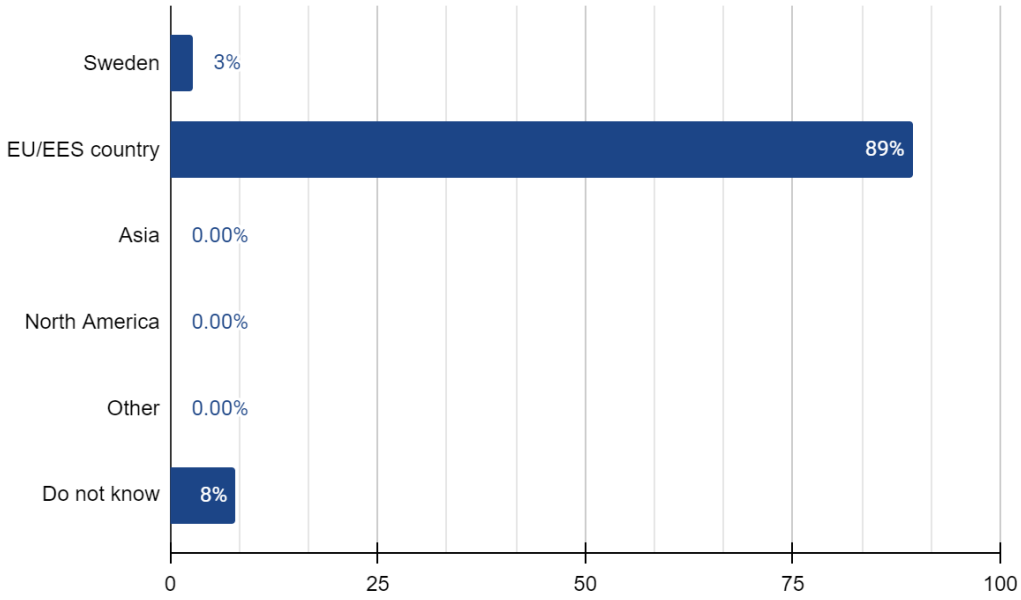

Figure 11. ´Question: Which area are the majority of the rats being purchased from at your facility?´

Several aspects can be important when choosing a breeder. Such aspects are the health status of the animals, animal husbandry at the breeder, the length of the transportation time for the animals between the breeder's facility and the research facility, the cost of the animals and animal handling at the breeder. The participants were asked to grade the importance of these factors when choosing a breeder. The grading scale ranged from 1-4, where 1 = Completely disagrees, and 4 = Completely agree. The animal's health status was very important for the majority of the participants where 92% chose 4 = Completely agree and 8% did not know. Animal husbandry showed more variation where 50% completely agreed that it was an important factor and the other 50% answered 2, 3 or Do not know. When asked if transportation time was an important factor, 5% completely disagreed and 39% completely agreed. 45% either chose 2 or 3. The cost was an important factor for 24% of the participants choosing 4, completely agree, and 5% chose completely disagree. A total of 45% of the participants completely agreed that animal handling at the breeder was an important factor and 3% completely disagreed, where 35% chose 2 or 3 in regard to animal handling being an important factor. Results can be seen in (Figure 12).

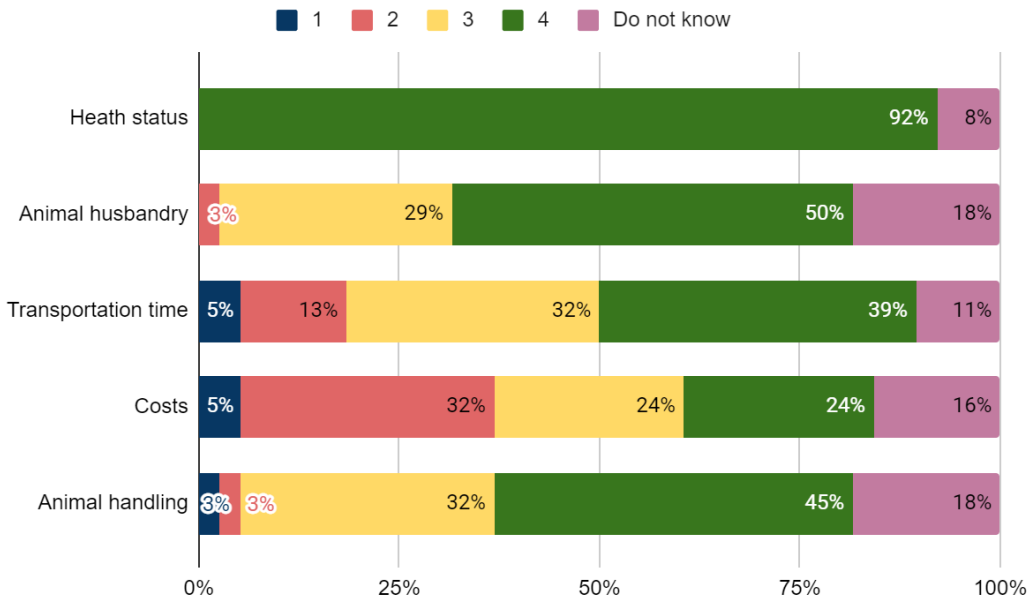

Figure 12. ´Question: Following are some factors that may affect your choice of supplier when purchasing rats for scientific experiments. Please grade the following statements from 1-4 based on how much each applies to the facility you work at. 1 = Completely Disagree and 4 = Completely agree.

19 participants reported that they acclimatise their rats for less than 7 days (<7). These 19 participants were also compared against each other when looking at other questions regarding transportation. When asked how many of their rats were purchased from a breeder, 63% of them answered that they buy 75-100% of their laboratory rats and all of them answered that the rats come from an EU- or EES country. Further, when asked if transportation time was an important factor when choosing a breeder, 37% rated it as a ´2´, 21% as ´3´, 32% as ´4´ and 11% as ´Do not know´.

A total of 17 participants reported that they acclimatise their rats for 7 days or more than 7 days (>7) and 59% answered that the majority of rats used at their facility came from a breeder. All of them answered that they buy the rats from an EU- or EES country. When asked if transportation time was an important factor when choosing a breeder, 6% rated it as ´1 = Completely disagree´, 18% rated it as a ´2´, 18% as ´3´, 35% as ´4 = Completely agree´ and 24% as ´Do not know´.

One participant in the survey answered that they do not acclimatise their rats before scientific experiments. The participant also stated that 0-25% of all rats came from a breeder and that the majority of rats that they do get from a breeder, came from a breeder within Sweden. No explanation was given by the participant if the rats they bought came from an external Swedish breeder or from another animal section within their own research facility.

A section of the survey included questions regarding the acclimatisation manual. Questions included asking who at the facility had the main responsibility to ensure

that the acclimatisation of the rats is implemented in accordance with the written acclimatisation manual and which person/s implement/s the acclimatisation of the rats at their facility. Both questions were multiple choice, and the participants had the option to tick as many or as few roles as needed. The role titles included Laboratory Animal Veterinarian, Animal Health Professional, Researcher, Laboratory Assistant, NACWO, and Animal Facility Manager.

The results showed that Researchers, Laboratory Animal Veterinarians and NACWO were the three groups that most often had the responsibility, or shared the responsibility, to make sure that the practical work to acclimatise rats at the research facility followed the acclimatisation manual. The largest group to have this responsibility included in their job was Researchers with 37%, followed by Laboratory Animal Veterinarians and NACWO at 16% respectively. 13% did not know who had this responsibility. When asked which person or persons usually work with the animals to acclimatise them, the majority (38% respectively) answer-red that Researchers or Animal Health Professionals acclimatise the animals.

These questions were then followed by asking how it is verified that the acclimatisation of the rats had been implemented in accordance with the acclimatisation plan. Below is a selection of answers. A full account of all answers can be viewed in Appendix 2. The answers have been translated from Swedish to English by the author.

It is documented in the experimental plan with the additional protocol.

The rats are calmer.

Logbook.

This is not verified independently and recorded for each animal. Breaches to our routine would be visible in the database.

We keep an eye on the researchers.

I ask the researchers working in my group to write this information in a lab book.

The acclimatisation manual (describing the acclimatisation time) is described in the ethical application and therefore statutory. Animal technicians will perform controls to make sure that researchers follow the manual.

Via a journal.

Do not know.

There are no controls.

The participants were also asked if there are procedures in place for if/when the acclimatisation is not carried out in accordance with the acclimatisation manual. A total of 53% did not know if there are any procedures in place if the plan is not followed, 26% answered that there were No procedures and 21% answered that they had procedures in place (Figure 13). A few participants who answered ´Yes´ added comments saying that if the procedures were not followed it would lead to an incident report, and/or an investigation or it would be reported to a supervisor.

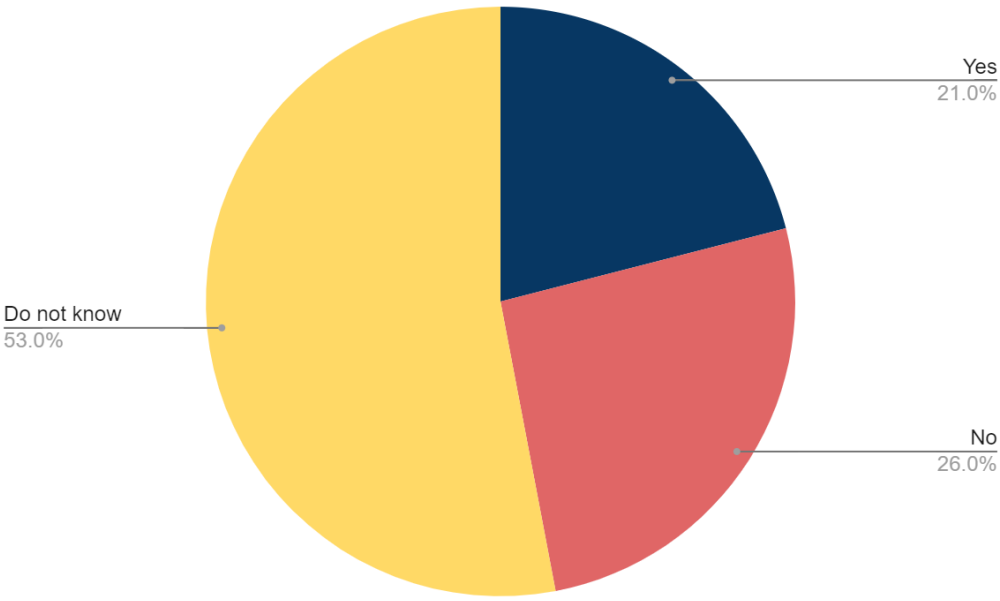

Figure 13. ´Question: Do you have procedures in place for if the acclimatisation is not carried out according to the acclimatisation plan?´

The last question of the survey asked if there were anything else the participant would like to add regarding the acclimatisation of rats. Below are quotes from three participants. Two quotes have been translated from Swedish to English by the author. A full account of all answers can be viewed in Appendix 2.

> *I believe that acclimatisation is an integral part of the 3R work and that the demands for acclimatisation/handling of the animals before scientific experiments, needs to be greater. With more accurate data and fewer animals required, all parties will benefit.*

> *In our facility, this is standard and not flexible, so there are no issues.*

I think it is essential to highlight the importance of acclimatisation and understand that it takes time. Animals with long transportation times and different living conditions at the breeder might need longer periods to acclimatise. I also believe that the people working with the animals, animal carers/researchers, need to spend more time handling the animals prior to the experiments.

5. Discussion

The focus of this study was to investigate how research facilities in Sweden formulate and conduct acclimatisation of rats before scientific experiments. This included; if any changes were made to the acclimatisation time based on the main purpose of the research, as well as examining if essential aspects of animal welfare would be taken into consideration when choosing a breeder. The survey results showed a widespread in acclimatisation time, ranging from 3 days to 2 weeks. A majority of everyone taking the survey reported an acclimatisation time shorter than 7 days and only 5% reported an acclimatisation time longer than 7 days. The literature is divided when recommending an appropriate acclimatisation time where some studies recommend 3-5 days (Capdevila *et al.* 2007; van Ruiven *et al.* 1998), while other studies recommend a longer period for rats to acclimatise after transport (Arts *et al.* 2012; 2014a; 2014b).

The difference in the recommendations can be due to several aspects. First of all, the conditions under which these studies were done are quite different. Capdevila *et al*. (2007) and van Ruiven *et al.* (1998) both transported their rats for a shorter period, as well as transporting them back to the same facility, but to a different animal room. Arts *et al.* (2012; 2014a; 2014b) transported the rats from a breeding facility to a new facility. The rats were also transported over a longer period which spanned at least two days. The longer time of transportation and the fact that the rats were transported to a different facility, is more representative for the conditions in many countries. van Ruiven *et al.* (1998) reported that the rats' corticosterone levels recovered rather quickly after transportation and three days acclimatisation time after transport was considered sufficiant. As acknowledged in van Ruivens *et al.* (1998), the blood collection was not made swiftly enough after transportation and was not done as reliably. This would indicate that acclimatisation following Arts *et al.* (2012; 2014a; 2014b) would be more accurate. The studies made on the acclimatisation of rats after transport, are few and give a wide range of recommendations for acclimatisation time. In regard to the acclimatisation time given at Swedish research facilities based on the results of the survey, therefore mirror the recommendations of the scientific studies which have been highlighted in this study, rather well.

Other aspects which need to be considered when deciding on the acclimatisation time is what other stressors the rats will go through at the facility. This may include moving within the facility before experiments and light-dark shifts. In the survey, when asked if further acclimatisation of rats took place when moved within the facility, a large number of respondents answered ´No´ or ´Do not know´.

Arts *et al.* (2014b) recommended a 2-day acclimatisation time after internal transferring of the rats within the research centre. Similar results were seen (Figure

8) when asked if the rats get acclimatised after light-dark shift, where the majority answered ´Do not know´. Not knowing if the rats get acclimatised after a light-dark shift could be due to the fact that changing the light-dark period is something that is not relevant in all studies and therefore the participant would not know if the animals would be acclimatised in these cases. However, 11% answered that they do not acclimatise the rats when changing the light-dark period.

Zangh *et al.* (2000) changed the circadian rhythm by extending the dark period by 4 hours before reverting it to a 12/12-hour cycle. The study showed that it took 1-3 weeks before the acrophase of the measured parameters returned to normal levels.

Moreover, van den Buuse (1999) did a similar study showing that it took between 3-5 days for heart rate, blood pressure and acrophase to stabilise. Even though these two studies show a different period in the time it took for the rats to acclimatise after a light-dark shift, the results still show a need for acclimatisation. The results from the survey show that the majority of the participants do acclimatise the rats after transport. However, acclimatisation within the facility after changes to the rat's environment such as re-grouping, re-location, or a shift in the circadian rhythm, is not as common or generally practised. There is a higher percentage of participants who have either no knowledge of further acclimatisation at their facility or the facilities seem to not always acclimatise the rats after such events. It needs to be mentioned that some participants who answered ´No´ or ´Do not know´ to any of the mentioned changes in this paragraph, added comments after their answers explaining that for example, their facility did not re-group the rats, the rats were held in one room from arrival to the end of the experiment or the facility never needed to change the circadian rhythm. However, some participants did not add any comments explaining if re-grouping, internal transfer or changes to the light-dark period took place. It is, therefore, open to speculation, if these participants facilities simply do not acclimatise the rats after changes within their environment or if the facilities simply do not need to change the rat's environment after arrival, thus further acclimatisation is not needed. The survey would therefore have benefitted if it had contained more questions regarding acclimatisation at different times and asked why or why not acclimatisation took place. Further investigation into the regulations at research facilities and their acclimatisation routines would also be necessary to draw any conclusions. The high percentage of participants answering ´No´ or ´Do not know´ to the questions regarding re-grouping, internal transfer and light-dark shift, shows that there is a need for more data showing the effects on rats after transportation or changes in the environment and recommendations for appropriate acclimatisation times.

When partaking in the survey, participants who stated that the rats are acclimatised were then given more questions regarding the acclimatisation manual at their research facility. One such question asked what their acclimatisation time

was based on. Approximately 25% said that the acclimatisation time used at their research facility was mainly based on experience and/or recommendations or to reduce stress in rats before the experiment, respectively. The majority therefor stated they were the main sources used when establishing and acclimatisation time for the rats. A smaller percentage or participants, 11%, based the time on scientific studies and 9% based it on legislation or LASA guidelines. This raises questions wondering why not more facilities base their acclimatisation times on evidence-based research, and the possibility is that the recommendations are few, as previously stated. In the Swedish statute regarding regulations on laboratory animals (SJVFS 2019:9), there is little information to be found concerning acclimatisation times for the animals. The same can be said for ETS No. 123 (2006). The information available is both scarce and ambiguous, this might be a factor as to why only 9% based their acclimatisation times on legislation or LASA guidelines. It shows that due to the lack of regulation in both the Swedish statute and ETS No. 123 (2006), it is difficult to use that to establish proper acclimatisation times. Since several scientific studies are also inconclusive with regards to the time needed after transport, the personnel working with laboratory animals do not have a great amount of material to use as a reference when deciding on appropriate acclimatisation.

In the survey, when asked what controls were carried out to make sure the acclimatisation manual was followed and what consequences it would include if they were not, the results showed that not all research facilities have any controls to ensure correct acclimatisation in accordance with the manual. Others reported established control systems during acclimatisation in the form of documentation in different ways such as journals, logbooks, and data programs. When further asked if there were any procedures in place for when/if the acclimatisation plan was not followed, only 21% reported that such procedures exist and less than ¼ of all the participants reported a control system to establish that the animals have had enough time to acclimatise to their new environment.

As studies have shown, stress can affect research results (Abbott *et al.* 2006). Here, one must ask what impacts this may have. If the effectiveness of the acclimatisation cannot be authenticated, how can it be verified that the data gathered from the experiment is not impacted or changed due to stress? This is a source of error that is not taken into account.

The survey included questions regarding important factors when choosing a supplier. Many agreed that the health status of the animals was an important factor. However, factors like animal husbandry, transportation from the breeder, costs, and animal handling had more diverse answers regarding importance. That health status would be an important factor for the majority of the participants is not unexpected, since poor health status could be a source of data error. Animal husbandry at the supplier, transportation, and animal handling could however be a factor as stressful

handling, long transportation times and more, could be a source for the need of a longer acclimatisation time.

ETS No. 123 (2006) states that longer transportation or an unsuitable transportation environment could be an added stress factor that needs to be taken into consideration. van Ruiven *et al.* (1998) reported temperature changes of more than 10°C during transport, and humidity changes ranging from 21-100%. In the Swedish statute regarding regulations on laboratory animals (SJVFS 2019:9), it states that the relative humidity in animal enclosures should be above 40%. The Swedish Board of Agriculture also states on their webpage that the temperature in cars transporting rodents should never go above 25°C or below -5°C (Jordbruksverket 2022).

Large differences in the environment could be a major stress factor. van Ruvien *et al.* (1998) reported that one rat had died during transport and hypothesised that it might have been caused by the large differences in temperature and humidity within the transportation cages. To be transported in such environments or for long times would be the basis for longer acclimatisation times upon arrival at the research facility. Standardising the environment when transporting the rats would reduce some of the stress the rats undergo and lessen the acclimatisation time. The environmental changes are not only limited to the transportation itself, but to the fact that the rats undergo a big change in environment when moving from supplier to research facility. This is an important aspect which needs to be taken into account, even though it may be difficult to measure how this will affect the animals.

This survey was aimed to gather more information regarding how the acclimatisation of rats is conducted at Swedish research facilities. The information gained has given us a good insight into acclimatisation in Sweden, however it does have its limitations.

The literature has made it evident that male and female rats need to be given different lengths of acclimatisation (Arts *et al.* 2014a; 2014b).

When going through the literature, it is evident that there is a difference between the sexes that needs to be considered when discussing acclimatisation time. Studies have shown that females have high corticosterone levels for a longer period after being exposed to a stressor (Figueiredo *et al.* 2002).

Recommendations by Arts *et al.* (2014a) were 1 week for males and 2 weeks for females. The difference in acclimatisation time was not a subject which was mentioned in the survey and therefore it is difficult to draw any conclusions since there is no data available from the survey.

The survey included a section where the questions were designed to gather information regarding transportation. In hindsight, more questions should have been included to establish the length of transport, transportation types, etc. The survey results provided us with information that most rats are bought from breeders that are not based in Sweden but in another EU or EES country. Conclusions can

therefore be drawn that the rats will have to be transported internationally. However, from the information gathered in the survey, no conclusions can be made regarding how long the average transportation time might be or if the animals are mostly transported via motor vehicles, aeroplanes, trains, or by ships. Further questions should have been added to the survey to gather information on how long the rats are transported on average. The results could then have been compared to the length of the acclimation time given after the animals arrive at the research facility. It is also of interest to know if male and female rats get different lengths of acclimatisation after transport. The survey could also have benefitted from questions aimed at further clarifying acclimatisation time. When asked if the research facilities acclimatised rats before experiments, one participant answered ´No´ but added that they do not get acclimatised to special conditions. This would need some further clarification to know if the rats get acclimatised at all when they arrive at the research facility or if they do not undergo any acclimatisation after environmental changes such as re-grouping. The same can be said for the participant who answered ´Do not know´ on the same question and then added the comment ´Both Yes and No´. Further information would be needed to draw any conclusions regarding the answer given.

The survey was created in collaboration with the author of "Acclimatisation of mice before scientific experiments" (Karlsson, in press), where equivalent research has been made on the acclimatisation of mice. Karlsson (in press) had similar results to the survey as can be seen herein. The survey response was, however, much greater regarding acclimatisation of mice than rats. Karlsson (in press) received 105 completed surveys compared to 38 fully completed answers regarding the acclimatisation of rats herein. The answer frequency mirrors the usage of rats and mice as laboratory animals in Sweden where mice are much more common as laboratory animals (Jordbruksverket 2020, Table 1). Karlsson (In press) had a much higher answer frequency from researchers (77%). The answer frequency from researchers for this survey regarding rats, was 50%. Other similarities in answers between the two different surveys was that the acclimatisation time greatly differed. Results showed that mice were acclimatised for 72 hours and up to 2-4 weeks after transport (Karlsson, In press). Karlsson (in press) also found that even though a majority stated that the animals get acclimatised after transport, fewer participants answered that the mice get acclimatised after re-grouping (27%) and after changes in the circadian rhythm (19%).

6. Conclusion

When starting the investigation regarding the acclimatisation of rats before scientific experiments, the hypothesis was that the acclimatisation process including planning, and purchasing of the animals, as well as the practical work during the acclimatisation, would differ greatly between research facilities in Sweden due to the lack of acclimatisation guidelines. It is evident that the majority of research facilities do have an acclimatisation process in place, however, this study has shown that there are inconsistencies between research facilities regarding the acclimatisation time after transport, as well as acclimatisation after making changes to the rat's environment such as regrouping, and moving within the facility, since it has been shown that acclimatisation in these instances is not always common practice. Only a small percentage used legislation or LASA-guideline as their main resource when deciding the acclimatisation period. This could be an indication that a major reason for acclimatisation times being so different between facilities could be the lack of guidelines. ETS No. 123 (2006) acknowledges that the time required for acclimatisation depends on several factors but never mentions the exact time needed. The Swedish statute regarding regulations on laboratory animals (SJVFS 2019:9), describes how to care for and protect laboratory animals in research facilities. There are minimal mentions of any required time needed for the acclimatisation of rodents (SJVFS 2019:9). If ETS No. 123 (2006) or the Swedish statute (SJVFS 2019:9) would include an agreed-upon acclimatisation time for any laboratory animal, it could lead to a minimum time required. If the minimum time for acclimatisation would become standard practice due to legislation, it could be applied to every laboratory animal regardless of the length of transportation, changes in living accommodations, light-dark changes etc. This could result in short acclimatisation periods as a standard for every laboratory animal or laboratory rodent and might end up not giving the appropriate time for the animals to adjust to the transportation and their new environment. However, ambiguous and unclear guidelines will result in great differences in acclimatisation before experiments which in turn can affect the research data. It’s important to standardise the research by standardising the experiment and the acclimatisation time before the experiment to get results with less or no bias. This study is an essential part of showing the importance of acclimatisation before scientific experiments to acquire accurate data. There is a need for more studies to be made in order to give a greater understanding of why acclimatising animals before an experiment is vital for the integrity of the data. Another major factor is the need to clarify the guidelines in place and help laboratory personnel to use appropriate acclimatisation times. This is not only beneficial from an animal welfare perspective, but will also help standardise the experiments and make it more accurate. This

does not mean that there should be a set minimum time for acclimatisation, as it could cause research facilities to not properly consider different kinds of stressors. From the survey, it is also evident that not all research facilities have a proper system in place to control if the acclimatisation manual has been followed or if the rats are acclimatised to their new environment. Implementing such a system into the organisation would help discover poorly acclimatised animals before they are taken into any experiments. Understanding different kinds of stressors, such as the effect of different transportation times, the changes in housing, and the stress implication when transferred in-house, will help when recommending appropriate acclimatisation of rats. This is an important topic that needs to be discussed and given more attention as to how it can affect both the animals involved and the outcome of the research. Comments given by participants at the end of the survey also highlighted the need for more research both to favour scientific accuracy but also animal welfare.

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Popular science summary

Millions of animals are used annually in the EU for scientific experiments (European Animal Research Association (2021). In Sweden, the three most commonly used animals are mice, zebrafish and rats (Jordbruksverket 2020 table 1). Every member state in the European Union has to follow laws established by the EU as well as its own country's statutes and legislation (European Commission 2022). In Sweden, the laws regarding animal welfare and handling of laboratory animals are rather strict and extensive compared to other EU countries (Sveriges lantbruksuniversitet 2021).

Even so, there is little information and regulations regarding the treatment of laboratory animals before scientific experiments. Research facilities may not have the possibility to breed their own rats in houses and there are many suppliers on the market that breed and supply rats for experimental purposes. This means that when rats are a necessity for the study, research facilities may buy rats from a supplier who is then transported from the breeder to the research facility. It is known that transportation is very stressful for the rats and will include several stressful elements. At the research facility, the rats will be unpacked and placed in a new and unfamiliar environment, with new smells, new animal carers, new feed etc. All of these factors will be a source of stress for the rats and they will need some time after the transportation to get used to their new environment, they need to acclimatise. Studies have shown that stress can affect an individual both physiologically and psychologically (Abelson *et al.* 2005, Swallow *et al.* 2005, Fleshner *et al.* 1998).

Physiologically stress causes hormones such as corticosterone to be released (Abelson *et al.* 2005). Corticosterone is a hormone which can have an effect on several bodily systems such as the immune system, lowering its function (Loeb & Quimby 1999). Stress can also cause a change in heart rate and body weight as well as show behavioural differences (Morton *et al.* 1985).

All of these parameters can be good markers to measure stress and to investigate the length of time needed for the parameters to return to normal in rats after transportation.

Studies have shown that there is an increase in corticosterone levels in rats after transportation (Shim *et al.* 2009). There is also evidence supporting that there is a difference between males and females where the females show higher levels of corticosterone for a longer period of time after transport and therefore will need a longer acclimatisation time after transport compared to male rats (Arts *et al.* 2014b). Studies have also shown that stress can affect the outcome of results. Abbott et al. (2006) showed that a known gastrointestinal hormone which admittedly has

anorectic tendencies in rats and humans is proven to have a lesser effect on the individual if they are stressed.

The recommended acclimatisation time based on literature is however divided. It ranges from 3-5 days up to almost 2 weeks in later studies. Some studies also recommend a 2-day acclimatisation time for rats who are moved within the research facility as that may also involve stress (Arts *et al.* 2014b).

In this paper, we have investigated how long the acclimatisation time is at different research facilities in Sweden, as well as looked into how the acclimatisation has been conducted. This has been done by sending out an anonymous digital survey, through the Swedish 3R-center to a category of people who work with rats as laboratory animals at different research facilities. The survey showed that the majority of research facilities in Sweden let their animals acclimatise to their new environment after transportation. However, the length of the acclimatisation time varied greatly some claimed to let their rats acclimatise for less than 7 days after transport, and some let them acclimatise for 7 days or more. The results also showed that a majority of facilities buy most of their rats from a breeder and few have in-house breeding. A majority of facilities also buy more than 50% of their rats from a breeder outside of Sweden. The survey also included questions asking what the participants based the length of the acclimatisation time on. More than 50% either based the time on experience/recommendations or the notion of stress reduction, i.e. the purpose was to reduce stress in the rats before experiments. Very few answered that their acclimatisation time was based on laws or legislation.

The results showed that most rats used in scientific research in Sweden do get acclimatised after transport. However, how long this acclimatisation time is and how and when it is conducted varies greatly. Short acclimatisation time or no acclimatisation time is not only an animal welfare problem but can also affect the accuracy of scientific data. The large differences in acclimatisation time could be due to the lack of regulations and guidelines, both in Article 5 of Appendix A of the European Convention but also within the Swedish statute regarding regulations on laboratory animals (SJVFS 2019:9). Neither include recommendations for specific acclimatisation times and are written with both ambiguities and are unclear on what is an appropriate acclimatisation time. It is therefore important to conduct more studies on the subject to get a greater understanding and standardisation of what an appropriate acclimatisation time is. As of now, the literature is also divided into its recommendations. There is also a need for clarification on what is recommended as an appropriate acclimatisation time in both the EU convention and the Swedish statute. However, a minimum time for acclimatisation written into the legislation might not be the best solution. This may in turn cause a large number of animals to only be acclimatised for the minimum amount of time instead of looking to the individual's needs for acclimatisation. The acclimatisation time should be based on scientific data and help guide laboratory personnel to decide on an

appropriate acclimatisation time based on transportation time, changes in the environment, and differences between sexes to name but a few. More studies are therefore needed on the subject. Helping rats and other laboratory animals get an appropriate acclimatisation time is not only good from a scientific point of view as it may help to standardise data, but it is also beneficial for animal welfare.

Acknowledgements

Thank you to my supervisors, Erika Roman and Katarina Cvek, for all your knowledge, expertise, help and enthusiasm throughout this dissertation. A big Thanks to Frida Karlsson, author of `Acklimatisering av möss inför försök`, for her support, hard work and inspiration. Thank you to Emelie Jansson at the Swedish 3R-center for her hard work and expertise on the subject and for also giving us a platform for our survey.

Finally, thank you to Sam Stewart-Keene for his linguistic and computer skills as well as his moral support throughout this dissertation.

Appendix 1

Digital survey

Enkät om acklimatisering av råttor / Survey about the acclimatisation of rats

English explanation follows below. Throughout this survey, explanations, questions and answers are first written in Swedish, and then followed by the same information in English.

Under hösten 2022 genomför Sveriges 3R-center tillsammans med SLU en enkätundersökning av hur acklimatisering planeras och genomförs på anläggningar i Sverige där råttor, möss och zebrafiskar hålls för forskning. I denna enkät ingår både frågor om eventuella skriftliga planer som finns och hur acklimatisering genomförs i praktiken. I enkäten undersöker vi också vilka aspekter som tas i beaktande vid val av leverantör av försöksdjur, eftersom detta potentiellt kan påverka djurens behov av acklimatisering inför försök. Resultaten kommer hjälpa Sveriges 3R-center att ge råd om hur dessa djurslag bör acklimatiseras vid olika händelser.

Denna enkät riktar sig till dig som kommer i kontakt med råttor i forskning. Arbetar du även med möss eller zebrafiskar är du välkommen att svara på enkäterna gällande dessa djurslag. Du bör ha fått länkar till dessa enkäter i ett utskick. Har du inte fått dessa, men önskar svara på enkäten, vänligen kontakta oss på 3Rcenter@jordbruksverket.se.

Varje enkät beräknas ta 10-15 minuter att besvara och alla svar är anonyma.
Enkäten består av flera sidor. När du svarat på alla frågor på en sida går du vidare genom att klicka på Nästa. När du har svarat på alla frågor klickar du på Slutför.

Tack för att du tar dig tid och svarar på denna enkät!

During the autumn of 2022, the Swedish 3R-center in collaboration with SLU are making surveys to investigate how acclimatisation of rats, mice, and zebrafish is planned and implemented at different research facilities across Sweden. This survey includes both questions about potential written procedures regarding acclimatisation, and how the acclimatisation is carried out in practice. The survey also explores the different aspects that might be taken into consideration when choosing a supplier, since this could affect the animals' needs of acclimatisation prior to trial. The results will help the Swedish 3R-centre to give advice regarding how these animals should be acclimated to a new environment depending on the circumstance.

This survey is primarily directed at people coming in contact with rats as research animals. If you also work with mice and zebrafish we appreciate it if you would take the time to fill out the surveys for these animals as well. You should have received links to all the surveys in an email. Should you want to answer the other surveys but do not have a link, please contact us at 3Rcenter@jordbruksverket.se

Each survey is expected to take 10-15 minutes to answer and all answers are anonymous.
The survey consists of several pages. When you have answered all the questions on one page, please click "Nästa", meaning next, to continue to the next page. When you have answered all questions, click "Slutför", meaning submit.

Thank you for taking the time to answer our survey!

Vilket av följande beskriver din nuvarande arbetsroll bäst?
Which of the following alternatives best describes your current role title?

- ○ Försöksdjursveterinär / Laboratory animal veterinarian
- ○ Försöksdjurstekniker eller djurvårdare / Animal health professional
- ○ Forskare / Researcher
- ○ Laboratorietekniker eller biomedicinsk analytiker / Laboratory Assistant
- ○ Föreståndare för djurens välbefinnande och skötsel / Named Animal Care and Welfare Officer (NACWO)
- ○ Djurhuschef / Animal facility manager
- ○ Annat / Other

Acklimatiseras råttor inför försök på din anläggning?
Are rats acclimatised at your research facility?

- ○ Ja / Yes
- ○ Nej / No
- ○ Vet ej / Do not know

Kommentar
Comment

Avbryt

1 / 11

Nästa

Finns det en skriftlig plan för acklimatisering av råttor inför försök på din anläggning?

Does your research facility currently have a written manual describing how the acclimatisation of rats should be conducted prior to trials?

- ○ Ja /Yes
- ○ Nej / No
- ○ Vet ej / Do not know

Kommentar

Comment

Avbryt Föregående

2 / 11

Nästa

Vilka typer av källor har använts för att utarbeta den skriftliga planen gällande acklimatisering av råttor? Vänligen välj alla korrekta svar.

Which types of sources have been used to develop the written manual regarding acclimatisation of rats? Please choose all options that apply.

- ☐ Lagstiftning / Legislation
- ☐ Vetenskapliga studier / Scientific studies
- ☐ Erfarenheter / Experience
- ☐ Vet ej / Do not know
- ☐ Annat / Other

Kommentar

Comment

Avbryt Föregående

3 / 11

Nästa

Acklimatiseras råttor vid ankomst till din anläggning?
Are rats acclimatised when first arriving at your facility?

- ○ Nej / No
- ○ Vet ej / Do not know
- ○ Ja, vänligen ange hur länge / Yes, please note for how long

Kommentar
Comment

Acklimatiseras råttor vid omgruppering på din anläggning?
Are rats acclimatised when they are re-grouped at your facility?

- ○ Nej / No
- ○ Vet ej / Do not know
- ○ Ja, vänligen ange hur länge / Yes, please note for how long

Kommentar
Comment

Acklimatiseras råttor vid flytt inom din anläggning?
Are rats acclimatised when they are moved to a new location within your facility?

- ○ Nej / No
- ○ Vet ej / Do not know
- ○ Ja, vänligen ange hur länge / Yes, please note for how long

Kommentar
Comment

Acklimatiseras råttor vid förändring av dygnsrytm på din anläggning?
Are rats acclimatised when the circadian rhythm is changed at your research facility?

- ○ Nej / No
- ○ Vet ej / Do not know
- ○ Ja, vänligen ange hur länge / Yes, please note for how long

Kommentar
Comment

Beskriv kortfattat hur du genomför acklimatisering av råttor.
Briefly describe how you acclimatise rats.

Vad ligger till grund för den acklimatiseringslängd som används för råttor?
What are the main reasons used when deciding the length of acclimatisation of rats?

Skiljer sig planen för acklimatisering av råttor åt beroende på försökets syfte, exempelvis om det är beteendestudie eller terminalförsök?
Does the plan for acclimatisation of rats change depending on the purpose of the research, for example if the purpose of the research is a behavioural study or a terminal trial?

- ○ Ja / Yes
- ○ Nej / No
- ○ Vet ej / Do not know

Kommentar
Comment

Avbryt | Föregående | 4 / 11 | Nästa

Vänligen beskriv vad det är som skiljer de olika acklimatiseringsplanerna för råttor åt beroende på försökets syfte.
Please describe the changes that are made to the acclimatisation process based on the purpose of the research.

Avbryt | Föregående | 5 / 11 | Nästa

Är planen för acklimatisering olika beroende på om råttor köps in från leverantör eller är uppfödda på anläggningen?

Is the plan for acclimatisation of rats different depending on if they were purchased from a supplier or if they were bred at your facility?

- ○ Ja / Yes
- ○ Nej / No
- ○ Vet ej / Do not know

Kommentar

Comment

Avbryt | Föregående | Nästa

6 / 11

Vad är det som skiljer de olika acklimatiseringsplanerna åt beroende på om råttor köpts från leverantör eller är uppfödda på anläggningen?

What changes in the acclimatisation plan depending on if the rats were purchased from a supplier or if they were bred at your facility?

Anpassas acklimatiseringen utifrån transportens längd när råttor köps in från leverantör?

Does the acclimatisation length change depending on transportation time when rats are purchased from a supplier?

- ○ Ja / Yes
- ○ Nej / No
- ○ Vet ej / Do not know

Kommentar

Comment

Avbryt | Föregående | Nästa

7 / 11

Ungefär hur stor andel av råttor som används på din anläggning har köpts in från leverantör?

Approximately what percentage of the rats that are used at your facility have been purchased from a supplier?

- ○ 75-100 %
- ○ 50-75 %
- ○ 25-50 %
- ○ 0-25 %
- ○ Vet ej / Do not know

Från vilket område införskaffas majoriteten av de råttor som köps in till din anläggning?

Which area are the majority of the rats being purchased from at your facility?

- ○ Sverige / Sweden
- ○ EU eller EES-land / EU or ESS country
- ○ Asien/ Asia
- ○ Nordamerika / North America
- ○ Vet ej / Do not know
- ○ Annat / Other

Nedan följer ett antal faktorer som kan påverka val av leverantör vid inköp av råttor. Vänligen gradera 1-4 beroende på hur viktiga faktorerna är för din anläggning. 4 = Mycket viktigt och 1 = Inte alls viktigt

Below are factors that can impact the choice of supplier when purchasing rats. Please grade how important the factors are for your facility. 1-4 based upon how much each applies to your facility. 4 = Very important and 1 = Not at all important

	4	3	2	1	Vet ej / Do not know
Hälsostatus / Heath status	○	○	○	○	○
Djurhållning / Animal husbandry	○	○	○	○	○
Transporttid / Transportation time	○	○	○	○	○
Kostnad / Costs	○	○	○	○	○
Djurhantering / Animal handling	○	○	○	○	○

Kommentar

Comment

Om ni undersöker leverantörens husering och hantering av råttor, hur går det till?

If you investigate the suppliers' housing and handling of the rats, how is it done?

Avbryt | Föregående | Nästa

8 / 11

Vem på din anläggning har huvudansvar för att säkerställa att acklimatiseringen av råttor genomförs enligt acklimatiseringsplanen?

Who at your facility has the main responsibility to ensure that the acclimatisation of rats is implemented in accordance with the acclimatisation plan?

- ○ Försöksdjursveterinär / Laboratory animal veterinarian
- ○ Försöksdjurstekniker eller djurvårdare / Animal health professional
- ○ Forskare / Researcher
- ○ Laboratorietekniker eller biomedicinsk analytiker / Laboratory Assistant
- ○ Föreståndare för djurens välbefinnande och skötsel / Named Animal Care and Welfare Officer (NACWO)
- ○ Djurhuschef / Animal facility manager
- ○ Vet ej / Do not know
- ○ Annat / Other

Vem eller vilka på din anläggning genomför acklimatiseringen av råttor? (Vänligen välj alla korrekta svar)

Who at your facility implement the acclimatisation of rats? (Please choose all options that apply)

- ☐ Försöksdjursveterinär / Laboratory animal veterinarian
- ☐ Försöksdjurstekniker eller djurvårdare / Animal health professional
- ☐ Forskare / Researcher
- ☐ Laboratorietekniker eller biomedicinsk analytiker / Laboratory Assistant
- ☐ Föreståndare för djurens välbefinnande och skötsel / Named Animal Care and Welfare Officer (NACWO)
- ☐ Djurhuschef / Animal facility manager
- ☐ Vet ej / Do not know

Hur kontrolleras att acklimatiseringen av råttor genomförs enligt acklimatiseringsplanen?

How do you verify that the acclimatisation of rats has been implemented according to the acclimatisation plan?

Finns det en handlingsplan för om acklimatiseringen inte skulle följa acklimisatiseringsplanen?

Do you have procedures in place for if the acclimatisation is not carried out according to the acclimatisation plan?

- ○ Ja / Yes
- ○ Nej / No
- ○ Vet ej / Do not know

Kommentar

Comment

Avbryt | Föregående | Nästa

9 / 11

Vad innebär handlingsplanen för när acklimatisering inte följer acklimatiseringsplanen?

What are the procedures for when acclimatisation has not been carried out according to the acclimatisation plan?

Avbryt | Föregående | 10 / 11 | Nästa

Är det något annat du vill att vi ska veta gällande acklimatisering?

Is there anything else you would like us to know about acclimatisation?

Avbryt | Föregående | 11 / 11 | Slutför

Tack för att du deltog i denna enkät.

Appendix 2

Survey answers and comments

1. Which of the following alternatives best describes your current role title?	
Researcher	19
Laboratory Assistant	4
Animal health professional	6
Laboratory animal veterinarian	5
Named Animal Care and Welfare Officer (NACWO)	3
Animal facility manager	1
Other	0

2. Are rats acclimatised at your research facility?	**Yes***	**No****	**Do not know*****
Researcher	18	1	0
Laboratory Assistant	4	0	0
Animal health professional	5	0	1
Laboratory animal veterinarian	5	0	0
Named Animal Care and Welfare Officer (NACWO)	3	0	0
Animal facility manager	1	0	0

** If Yes, the participant proceeded to question 3.*
*** If No, the participant proceeded to question 16 and did not have the possibility to answer questions 3-15.*
**** If Do not know, the participant proceeded to question 16 and did not have the possibility to answer question 3-15.*

Comments:
Our rats are acclimatised for 5 days
We work with diabetes. It is very important that our rats feel safe with whoever performs the experiment
Both Yes and No
We do not acclimatise to special conditions
Yes and No, it is different between studies. For a PK study, we catheterize the rats, then No. For a longer inhalation study where the rats are in specific cages, then Yes.
At least 5 days

3. Does your research facility currently have a written manual describing how the acclimatisation of rats should be conducted prior to trials?	**Yes***	**No****	**Do not know****
Researcher	6	3	9
Laboratory Assistant	1	1	2
Animal health professional	4	1	0
Laboratory animal veterinarian	4	1	0
Named Animal Care and Welfare Officer (NACWO)	2	1	0
Animal facility manager	0	1	0

** If Yes, the participant proceeded to question 4.*
*** If No, the participant proceeded to question 5 and did not have the possibility to answer question 4.*
**** If Do not know, the participant proceeded to question 5 and did not have the possibility to answer question 4.*

Comments:
I'm at a new workplace and have not been introduced to such a plan. Based on the directive I assume that they have one, I will however instead base it on if there is a need for it in my experiments.
In our ethical application, we will state the minimum time for acclimatisation before behavioural studies.
Each research group will decide for themselves
We have our own plan
It is part of the animal protection organisations policy

4. Which types of sources have been used to develop the written manual regarding acclimatisation of rats? Please choose all options that apply.	**Legislation**	**Scientific studies**	**Experience**	**Do not know**	**Other**
Researcher	4	0	0	2	0
Laboratory Assistant	1	0	0	0	0
Animal health professional	3	0	0	1	0
Laboratory animal veterinarian	1	2	1	0	0
Named Animal Care and Welfare Officer (NACWO)	1	0	1	0	0
Animal facility manager	0	0	0	0	0

5. Are rats acclimatised when first arriving at your facility?					
	No	Do not know	<7 days	7 days	>7 days
Researcher	0	0	7	11	0
Laboratory Assistant	0	0	4	0	0
Animal health professional	0	0	3	1	1
Laboratory animal veterinarian	0	0	4	1	0
Named Animal Care and Welfare Officer (NACWO)	0	0	1	2	0
Animal facility manager	0	0	0	0	1

Comments:
5 days
5 days according to our ethical application. Not sure if this is standard at the facility.
At least 2 weeks of acclimatisation before the study begins.
(Answered 5 days for acclimatisation time). With the exception of animals with certain types of catheters since they may stop working. For these animals, an exception can be made and the acclimatisation time may only be 24h after arrival.
(Answered 5 days for acclimatisation time). The acclimatisation time should be prolonged if the transport was long or the animals experienced delays or bad weather. Rats with catheters will have a shorter acclimatisation time as the catheters may not last as long.
(Answered 5 days for acclimatisation time for terminal experiments and 7 days for chronic experiments). In some cases (some acute experiments where the animals do not need to be calm), the acclimatisation time may be skipped.

6. Are rats acclimatised when they are re-grouped at your facility?			
	Yes	No	Do not know
Researcher	2	3	13
Laboratory Assistant	0	3	1
Animal health professional	0	5	0
Laboratory animal veterinarian	0	3	2
Named Animal Care and Welfare Officer (NACWO)	0	2	1
Animal facility manager	0	1	0

Comments:
I do it in my experiments, however, I do not know if everyone does. The length might vary if the rats are adolescents or adults, depending on how the experiment is designed. Adult rats get 7 days if they stay on the same circadian rhythm.
Not applicable
It probably varies between researchers
Our rats usually get to socialise in big observational boxes so they already recognise each other.
Not applicable/cannot speak for facility
We do 2 to 4 days, with regular observations by the experimenter to make sure rats don't fight.
We have our own plan for re-grouping
We very rarely re-group our rats other than after arrival
Usually not, however, it may happen. It depends on the experiment.

7. Are rats acclimatised when they are moved to a new location within your facility?			
	Yes	No	Do not know
Researcher	2	0	16
Laboratory Assistant	0	0	4
Animal health professional	1	4	0
Laboratory animal veterinarian	0	2	3
Named Animal Care and Welfare Officer (NACWO)	0	3	0
Animal facility manager	0	1	0

Comments:
In my experiments, this is not applicable. We acclimatise the rats to the different rooms where the experiments will take place.
Some kind of acclimatisation should take place after re-location, however, I am not sure.
We only have one department at our facility
Not applicable/cannot speak for the facility
The moves are very small and should not disrupt or stress the animals. Normally they do not need to be acclimatised.
We have our own plan
Normally, No, but it may happen. It depends on the experiment.

8. Are rats acclimatised when the circadian rhythm is changed at your research facility?			
	Yes	No	Do not know
Researcher	6	0	12
Laboratory Assistant	0	0	4
Animal health professional	0	2	3
Laboratory animal veterinarian	2	0	3
Named Animal Care and Welfare Officer (NACWO)	1	2	0
Animal facility manager	0	0	1

Comments:
14 days if the circadian rhythm is changed by 12h.
If we change the circadian rhythm we usually acclimatise for 14 days before behavioural studies.
Several days but would need to ask to be sure
It is the same circadian rhythm in all animal rooms.
Not applicable
If it's only a few hours, for example, change to/from summertime/wintertime, we make sure they are not involved in any behavioural studies in direct proximity to the change. If the circadian rhythm is changed by 12 h, we try to acclimatise the rats for 14 days. However, this is not always possible straight after weaning and will depend on the experiment since they may "pass the teen stage".
So far we have not had to change the circadian rhythm
Not applicable. Cannot speak for the facility.
The circadian rhythm is the same all year round so no acclimatisation is needed
This is something the researcher will decide and not something that the facility demands
We have our own plan
This may vary and is not particularly common
We have not had this kind of experiment

9. Briefly describe how you acclimatise rats
We leave them undisturbed so that the physiological processes will have time to acclimatise
We make them accustomed to getting picked up and handled to minimise stress during the experiment
We let them be in their cages in the new animal room
It depends on the animal's purpose. If they're supposed to be used in a behavioural study they will be acclimatised upon arrival for a minimum of 1 week. After that, we "handle" the for 3-5 days (the researcher picks them up, allows them to climb around etc.) and transport them to the room where the experiments will take place in the same way as we would on the first day of the experiment.
They have a 12:12 light-dark cycle with fixed humidity and regular food. We do not disturb them other than to change bedding and top up their food
We move the rats 2h to the behavioural room before the experiment/test.
My rats get acclimatised "automatically" since they never move rooms unless there is an experiment in another room. During an experiment, they are allowed in the new room approximately 1h before the experiment will begin.
A lot of handling. We let them acclimatise when it includes taking a blood sample. Then we train the rats to be fixated.
We don't do anything with the animals.
The rats will be left undisturbed as much as possible during the 5 days after arrival to the facility, this also includes supervision and cage change. Some of the old enrichment/interior from the old cages will be moved into the new cages during the unpacking stage.
We handle/train the animals at least 5 times during the 2 weeks of acclimatisation. Gentle and calm handling will prepare the animals before the experiment.
The researchers are not allowed to do any experiments on the rats until 7 days have passed. However, they are allowed to come in and spend time with the rats so they get used to the researchers and their smell.
Upon arrival, the breeder will put the animals in their new cages. The only allowed interaction is handling, cuddles, and in soma cases if their tails need to be marked with a pen. Otherwise, they only get supervision, water, etc. Animals arriving on a Thursday will get a cage exchange at the same time as other animals, i.e. at the beginning of the following week.
They will be moved to their home cages. Some experiments include handling so the animals will get used to the experiment.
After arrival, they get 5 days of only supervision.
They will be left alone in their cages without intervention, potentially, some training involving handling may be done from day 2
The animals will acclimatise to their environment as well as feed/water. Training can start.

They will be caged in the same manner as other rats in the building. Access to wood wool and a stick to gnaw on. During this period, only handling and weighing the animals are allowed.
The rats will be placed in big, enriched cages and will be allowed to get used to the new environment. Stress-free handling "taming" is included in the acclimatisation time and can look different between groups.
The animals will be in their rooms with regulated light and dark period which is supposed to correspond to day and night. The animals will be handled daily in a way to habituate them. There is also a radio with a timer that corresponds to the same times as the lighting.
The animals will be placed in their enriched home cages in peace and quiet for 7 days
The rats will get a new feed which they will then acclimatise to. Then they will get daily supervision and also be picked up and handled to get used to the personnel.
Handling every day.

10. What are the main reasons used when deciding the length of acclimatisation of rats?

Experience/recommendations	9
Stress reduction	9
Routins at the research facility	3
Other	2
Scientific studies	4
Legislation/LASA	3
Health	1
Do not know	4

11. Does the plan for acclimatisation of rats change depending on the purpose of the research, for example if the purpose of the research is a behavioural study or a terminal trial?

	Yes *	**No****	**Do not know*****
Researcher	6	9	3
Laboratory Assistant	2	1	1
Animal health professional	2	2	1
Laboratory animal veterinarian	2	3	0
Named Animal Care and Welfare Officer (NACWO)	0	3	0
Animal facility manager	0	1	0

** If Yes, the participant proceeded to question 12.*

*** If No, the participant proceeded to question 13 and did not have the possibility to answer question 12.*

**** If Do not know, the participant proceeded to question 13 and did not have the possibility to answer question 12.*

Comments:
The physiological processes are just as important independent of the purpose of the research
Not in our facility, we normally acclimatise the rats for 7 days
We do not do behavioural studies
We believe that handling before the start of the experiments is more important before a behavioural study and emergency experiments than before chronic studies. Depending on what measures will be taken, the handling of chronic studies may not start before the first measurements for the experiments have started
We have the same acclimatisation for all our studies, however, some details may differ. If blood will be collected from the rat's tail. the tail will be handled more, if they will get an oral dose, this will be practised more after the 5 regular training occasions.
At least 5 days except for certain catheters. Sometimes it's longer.
Sometimes specific acclimatisation might be written into the ethical approval before a behavioural study.
Terminal experiments may sometimes be taken straight away.

12. Please describe the changes that are made to the acclimatisation process based on the purpose of the research.
Before behavioural studies, we handle the animals for several days before hand, in comparison to any terminal studies.
Behavioural studies need calm animals while terminal studies and operations do not have the same needs.
For emergency experiments, we only house the rats for a minimum of 5 days. During chronicle experiments, the rats will be weighed and handled for at least 7 days.
It may take up to 8 weeks to accomplish a baseline standard. Especially with young animals. But this depends on the research topic, e.g. behaviour, endocrine mechanisms and biorhythmic.
Before behavioural studies, greater habituation can be conducted towards the experimental manager as it may be good for the project.
Terminal experiments 5 days, chronicle experiments 7 days. In some cases, there is no acclimatisation at all (rare).
It's dependent on if the handling will start before or after the first experimental measure has started.
If we will only study anatomy, we will do no acclimatisation at all (the animals still need to be at the department for5 days before we sacrifice them.
It depends on which experiments the animal will be a part of and if they need to be at a specific weight or if they need to be extra calm etc.
We will train the rats for the specific tests they will endure, ex blood sampling, tube feeding
Terminal experiments may be taken earlier, otherwise, there are at least 5 days of acclimatisation as a standard.
Readouts such as blood glucose which are highly sensitive conditions, always require a longer acclimatisation time, while a normal PK has 5 days acclimatisation time which is deemed to be satisfactory

13. Is the plan for acclimatisation of rats different depending on if they were purchased from a supplier or if they were bred at your facility?

	Yes	No	Do not know
Researcher	4	3	11
Laboratory Assistant	2	1	1
Animal health professional	3	2	0
Laboratory animal veterinarian	3	2	0
Named Animal Care and Welfare Officer (NACWO)	1	2	0
Animal facility manager	0	1	0

** If Yes, the participant proceeded to question 14.*

*** If No, the participant proceeded to question 16 and did not have the possibility to answer questions 14-15.*

**** If Do not know, the participant proceeded to question 16 and did not have the possibility to answer questions 14-15.*

Comments:
I only use animals from approved breeders.
Not applicable
We usually always buy from a breeder
We have no breeding capabilities
We have never used rats breed at our facility
We do not breed rats
This only applies to animals arriving at our facility
We only buy from a breeder
No acclimatisation for self breeding
We do not breed rats.
The acclimatisation is only for transported animals. If they have been bred at the facility they are already accustomed to the environment.
Own-bred rats will be moved from one room to another and therefore do not need to acclimatise. It is equivalent to moving an animal from an animal room to the lab, no acclimatisation time is needed.
We do not engage in any breeding ourselves, but there would be a difference if we did.
Very little breeding of rats internally
We do not breed rats
We acclimatise when we buy from a breeder but not within the facility
Yes, if they are born in the facility acclimatisation is not needed

14. What changes in the acclimatisation plan depending on if the rats were purchased from a supplier or if they were bred at your facility?
I presume that the rats that are born at the facility can be used in experiments straight away
The animals which are not bought from a breeder will not be acclimatised in my understanding
My animals will be in the same room from birth and through the whole experiment
When transported from the facility the rats need to be at the department for 5 days before anything can be done with them
The same feed, water and enrichment for the cages are used through out the whole facility, acclimatisation will only be applicable for the animals which come from a breeder
5 days for animals from the breeder and non for own bred animals
The acclimatisation is only for transported animals. If they have been bred at the facility they are already accustomed to the environment.
No particular acclimatisation period for animals breed at the facility
Generally, we advise longer acclimatisation when animals are brought in from vendors.
There is no transport
Do not know
No acclimatisation within the facility
Animals from a breeder are not used to our routines/feed/water etc, they need to acclimatise

15. Does the acclimatisation length change depending on transportation time when rats are purchased from a supplier?			
	Yes	**No**	**Do not know**
Researcher	0	3	1
Laboratory Assistant	0	1	1
Animal health professional	0	3	0
Laboratory animal veterinarian	1	2	0
Named Animal Care and Welfare Officer (NACWO)	0	1	0
Animal facility manager	0	0	0

Comments:
The acclimatisation period is 7 days regardless of the length of the transportation
It is reasonable to extend the period if the transport is complicated
Rats transported from the USA or animals that seem more stressed upon arrival can get an extended acclimatisation time. This will be decided by the Laboratory Animal Veterinarian and the Research Leader.

16. Approximately what percentage of the rats that are used at your facility have been purchased from a supplier?					
	0-25%	**25-50%**	**50-75%**	**75-100%**	**Do not know**
Researcher	1	1	2	10	5
Laboratory Assistant	1	0	0	0	3
Animal health professional	0	0	0	6	0
Laboratory animal veterinarian	0	0	0	5	0
Named Animal Care and Welfare Officer (NACWO)	1	0	0	2	0
Animal facility manager	1	0	0	0	0

17. Which area are the majority of the rats being purchased from at your facility?						
	Swedeb	**EU/EES country**	**Asia**	**North America**	**Other**	**Do not know**
Researcher	1	16	0	0	0	2
Laboratory Assistant	0	3	0	0	0	1
Animal health professional	0	6	0	0	0	0
Laboratory animal veterinarian	0	5	0	0	0	0
Named Animal Care and Welfare Officer (NACWO)	0	3	0	0	0	0
Animal facility manager	0	1	0	0	0	0

18. Below are factors that can impact the choice of supplier when purchasing rats. Please grade how important the factors are for your facility. 1-4 based upon how much each applies to your facility. 4 = Very important and 1 = Not at all important					
A. Health status	**1**	**2**	**3**	**4**	**Do not know**
Researcher	0	0	0	17	2
Laboratory Assistant	0	0	0	4	0
Animal health professional	0	0	0	5	1
Laboratory animal veterinarian	0	0	0	5	0
Named Animal Care and Welfare Officer (NACWO)	0	0	0	3	0
Animal facility manager	0	0	0	1	0

B. Animal husbandry	1	2	3	4	Do not know
Researcher	0	0	6	10	3
Laboratory Assistant	0	0	1	3	0
Animal health professional	0	0	0	4	2
Laboratory animal veterinarian	0	0	1	2	2
Named Animal Care and Welfare Officer (NACWO)	0	1	2	0	0
Animal facility manager	0	0	1	0	0

C. Transportation time	1	2	3	4	Do not know
Researcher	0	3	7	6	3
Laboratory Assistant	0	0	2	2	0
Animal health professional	1	0	1	3	1
Laboratory animal veterinarian	0	1	2	2	0
Named Animal Care and Welfare Officer (NACWO)	1	1	0	1	0
Animal facility manager	0	0	0	1	0

D. Costs	1	2	3	4	Do not know
Researcher	1	6	4	5	3
Laboratory Assistant	0	1	2	1	0
Animal health professional	1	0	1	3	1
Laboratory animal veterinarian	0	2	1	0	2
Named Animal Care and Welfare Officer (NACWO)	0	3	0	0	0
Animal facility manager	0	0	1	0	0

E. Animal handling	1	2	3	4	Do not know
Researcher	0	0	6	10	3
Laboratory Assistant	0	0	2	2	0
Animal health professional	1	0	0	4	1
Laboratory animal veterinarian	0	0	2	0	3
Named Animal Care and Welfare Officer (NACWO)	0	1	1	1	0
Animal facility manager	0	0	1	0	0

Comments:
I can not answer for the research facility as a whole but only for my own reasoning in regard to the supplier. With the exception of what is listed above, the purpose of the research may sometimes be a factor as well.
I can not answer for the facility, only for my own group
We usually want animals from the same breeder to minimise potential differences between the animals in different experiments. However, I do not have the best knowledge about health status and animal husbandry. Our Laboratory Animal Veterinarian and Animal Health Professionals know more about that
As a research group we follow the advice from the facility.
In the question above you have forgotten to add an important perspective. The choice of supplier is mostly based on who has the correct strain/line the scientific question demands. All suppliers have different strains.
I don't have detailed knowledge about the choice of a supplier as that is something that is mainly made by other personnel. The statements above are based on my knowledge of it.

19. If you investigate the suppliers' housing and handling of the rats, how is it done?
Contact the supplier
Contact purchasing manager who in turn will contact the supplier
Do not know
Talk to a representative
We make a site visit
Do not know
We ask questions about animal husbandry and request pictures if possible
Not as an individual researcher. This is handled by the facility.
Audit visit
Normally through e-mail. They will send their instructions and sometimes video clips.
Do not know
Site-visit
We visit the supplier but we also have to trust their word and check their program for handling/animal husbandry and so on. No supplier will allow external visits due to the risk of infection
Discuss with colleagues and their experience with different suppliers.
If you visit the site you can get an understanding of animal husbandry or the handling of animals. Otherwise, you can ask the delivery representative when they visit our facility.
The Laboratory Animal Veterinarian sometimes visits the CRO and breeder
The board will visit the supplier

20. Who at your facility has the main responsibility to ensure that the acclimatisation of rats is implemented in accordance with the acclimatisation plan?

	Laboratory animal veterinarian	Animal health professional	Researcher	Laboratory Assistant	Named Animal Care and Welfare Officer (NACWO)	Animal facility manager	Do not know
Researcher	3	1	8	0	3	0	4
Laboratory Assistant	0	0	1	1	0	1	1
Animal health professional	1	1	1	0	1	2	0
Laboratory animal veterinarian	2	1	2	0	0	0	0
Named Animal Care and Welfare Officer (NACWO)	0	0	1	0	2	0	0
Animal facility manager	0	0	1	0	0	0	0

21. Who at your facility implement the acclimatisation of rats? (Please choose all options that apply)

	Laboratory animal veterinarian	Animal health professional	Researcher	Laboratory Assistant	Named Animal Care and Welfare Officer (NACWO)	Animal facility manager	Do not know
Researcher	4	11	15	1	2	1	1
Laboratory Assistant	0	1	2	2	0	1	1
Animal health professional	0	6	1	0	0	0	0
Laboratory animal veterinarian	0	4	4	0	0	0	0
Named Animal Care and Welfare Officer (NACWO)	0	2	2	1	1	0	0
Animal facility manager	0	1	1	0	0	1	0

22. How do you verify that the acclimatisation of rats has been implemented according to the acclimatisation plan?
There are no controls.
It is documented in the experimental plan with the additional protocol.
The rats are calmer.
Logbook.
This is not verified independently and recorded for each animal. Breaches to our routine would be visible in the database.
We keep an eye on the researchers.
I ask the researchers working in my group to write this information in a lab book.
The acclimatisation manual (describing the acclimatisation time) is described in the ethical application and therefore statutory. Animal technicians will perform controls to make sure that researchers follow the manual.
Via a journal.
We whom I listed above will together agree on the most optimal acclimatisation based on the purpose of the research, choice of supplier, length of transport etc
No idea
No extra controls
We have a sign on the cage that the rats are not to be used
Through the experimental plan
It is a close contact between Named Animal Care and Welfare Officer, researcher and director
No routines for this
Random samples for general control of compliance with other legislation, internal procedures, etc
We keep records of the training which are inserted into the experiment folder

Not sure. It is simply a procedure that we must follow like every other procedure.
Random samples by NACWO
No special control plan. Animal Technicians normally react if any deviations are noted.
Welfare and health parameters
Electronic system for study planning
The rats are not released before acclimatisation is complete.
When ordering animals, experiments must be planned in a system. This system only allows actions to be taken after the acclimatisation time has passed.
Through our journal system
Supervise the room. Animal Technicians check if the animals have been out of the room.
Several participants answered Do not know
Some participants left this question unanswered

23. Do you have procedures in place for if the acclimatisation is not carried out according to the acclimatisation plan?

	Yes*	No**	Do not know***
Researcher	3	3	13
Laboratory Assistant	0	1	3
Animal health professional	1	3	2
Laboratory animal veterinarian	2	2	1
Named Animal Care and Welfare Officer (NACWO)	2	1	0
Animal facility manager	0	0	1

** If Yes, the participant proceeded to question 24.*

*** If No, the participant proceeded to question 25 and did not have the possibility to answer question 24*

**** If Do not know, the participant proceeded to question 25 and did not have the possibility to answer question 24.*

Comments:

Most likely an incident report will be written
I think it would be impossible for us not to follow the accilmitisation because we are not allowed access until it is complete.
The Animal Technician will contact the nearest supervisor, alternatively, the Laboratory Animal Veterinarian will contact the Researcher in charge
If there are reasons to deviate from the routine, the Laboratory Animal Veterinarian will be contacted and may discuss the matter with the researcher.
It will be reported into a system which will then lead to an investigation

24. What are the procedures for when acclimatisation has not been carried out according to the acclimatisation plan?

It depends on the degree of deviation from the acclimatisation plan
Most likely an incident report will be written
Documentation and educational meetings
Adress this to the researcher in charge
The start of the experiment is postponed and extra acclimatization needs to be performed.
An investigation will be conducted regarding what and why, in order to determine measures that prevent this from happening again.
the matter is reported to the person in charge who then contacts the researcher. The conversation will lead to a remark, several remarks lead to suspension for a period of time and a requirement for further training.
It depends on the degree of deviation from the acclimatisation plan

25. Is there anything else you would like us to know about acclimatisation?

I believe that acclimatisation is an integral part of the 3R work and that the demands for acclimatisation/handling of the animals before scientific experiments, needs to be greater. With more accurate data and fewer animals required, all parties will benefit
In our facility, this is standard and not flexible, so there are no issues.
I think it is essential to highlight the importance of acclimatisation and understand that it takes time. Animals with long transportation times and different living conditions at the breeder might need longer periods to acclimatise. I also believe that the people working with the animals, animal carers/researchers, need to spend more time handling the animals prior to the experiments
No
Very strange questions in this survey. Many were not relevant to me as a researcher.

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