



Sveriges lantbruksuniversitet
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A study on Swedish researchers' attitudes and views of the 3Rs

En studie om svenska forskares attityder och åsikter om 3R-principen

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Master's thesis • 30 credits

Animal Science - Master's programme

Uppsala 2019

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Credits: 30 credits
Level: Second cycle, A2E
Course title: Independent project in Animal Science
Course code: EX0870
Programme/education: Animal Science - Master's programme
Course coordinating department: Department of Animal Breeding and Genetics

Place of publication: Uppsala
Year of publication: 2019
Online publication: <https://stud.epsilon.slu.se>

Keywords: 3R, attitudes, animal welfare

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Abstract

Every year, millions of vertebrates are used worldwide in experimental studies. If, how and when animals are allowed to be used in research is stated in the European directive 2010/63, incorporated in the Swedish animal welfare act (1988:534). In short, you are only allowed to conduct experiments with animals if; they cannot be replaced with any other method, the number of animals are reduced as much as possible (without compromising the potential results) and refinement has been applied to housing, procedures and care. To be allowed to work with experimental animals researchers must undergo proper education and, before any experiment begins, apply for an ethical permit from a regional animal ethics committee. In 2014 the Swedish Board of Agriculture was assigned by the Swedish government to form a centre for the 3Rs. To find out more about what researchers working in Sweden know about and implement when it comes to the 3Rs, the centre put together a survey which was sent to all researchers in Sweden who had applied for an ethical permit, during the period 2016-09-01 to 2017-08-31. Out of 555 individuals that could potentially answer the survey, 215 replied. With the help of the collected data from the survey this master's thesis aimed to find out more about the respondents attitudes to the 3Rs in relation to their background, how often they think about and discuss the subject, and find out what they consider to be obstacles/reasons for implementing the 3Rs. Two questions from the survey were chosen to represent if the respondents had a positive or negative attitude to the 3Rs. If they disagreed with the statements in the questions they were considered to have a more positive attitude and if they agreed, a more negative attitude. The results did not show any clear connection between the respondents' background and their attitudes to the 3Rs. Comparability with previous data and lack of appropriate scientific or technological innovation was considered to be two important obstacles for implementation of the 3Rs and ethical reasons was considered the foremost reason for implementation. The respondents seem to discuss refinement the most and half of them do not see any obstacles for its implementation.

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1. Introduction

1.1. The definition of an experimental animal

Animal experiments have been very beneficial to humans, providing us with knowledge, medicine and surgical skills, which prolong our lives. It has also been beneficial to animals, in the sense that it has led to breakthroughs in veterinary medicine as well. However, the high price for these potential benefits has been paid with the lives and suffering of the animals involved (Franco & Olsson, 2014). The Swedish definition of what constitutes an experimental animal is regulated in 1b§ in the Swedish animal welfare act (1988:534). It states that, “an experimental animal is any animal that is being used or is intended to be used in research. An experimental animal is also any animal that has been used or had been intended to be used in research and has been kept at a research facility, or similar, intended for experimental animals” (free interpretation into English). In Sweden the purpose dictates whether or not an animal is classified as an experimental animal.

The Swedish definition differs from the European definition where EU directive 2010/63 (article 3) states that an experimental animal is any animal that is included in procedures “which may cause the animal a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice. This includes any course of action intended, or liable, to result in the birth or hatching of an animal or the creation and maintenance of a genetically modified animal line in any such condition”. According to the EU, the procedure dictates whether or not an animal is classified as an experimental animal.

The consequence of this is that there is a higher number of animals that fall under the Swedish definition of experimental animals than there is according to the EU definition, since the latter definition excludes animals in behavioural studies, surveillance fishing (Swedish: provfiske), animals that are euthanized without any preceding procedure, and more (Ljung & Bornestaf, 2018). Before conducting experiments which includes animals you need to apply for an ethical permit, however, the Swedish and European legislation only includes animals that belong to the vertebrate category or to the class *Cephalopoda* (squid, octopus and cuttlefish) (Swedish animal welfare act [1988:534], Directive 2010/63/EU). Research performed on any species not belonging to these two groups does not require any ethical permit.

In Sweden for the year 2016, there was a reported use of over 350 000 experimental animals, according to EU definition (Ljung & Bornestaf, 2018). The majority of these were used in basic science (81 %) and the species used most were mice (75 %). During the same year, there was a reported use of over 6.5 million animals, which did not fall under the definition of EU. The majority of these were part of surveillance fishing (6 411 934 individuals) and markings of fish (152 214 individuals). A total of 90 % of the research performed on animals in Sweden this year were conducted at universities, colleges and at county councils (Swedish: Landsting) (Ljung & Bornestaf, 2018). In comparison, in Great Britain for the year 2016 there were 3.94 million procedures carried out on living animals (Home Office, 2017). The report by the Home Office (2017) states that the number of procedures performed corresponds quite well with the number of animals used. Approximately half of the procedures were part of experimental studies and the other half were for the creation of genetically modified animals (Home Office, 2017). Worldwide, between 50 and 100 million animals (vertebrates) are used in experimental studies every year (Würbel, 2017).

1.2. Severity categories

To get a better understanding of what animals in research may go through one can look at the different severity categories for experiments performed on animals. Annex VIII in EU directive 2010/63 lists the following four categories:

1. Non-recovery (The procedures are performed entirely under general anaesthesia and the animals do not recover consciousness afterwards)
2. Mild (The animals are likely to experience mild, short-term pain, suffering or distress from the procedure. Further, there should be no significant impairment of the well-being or general condition of the animals)
3. Moderate (The animals are likely to experience moderate, short-term pain, suffering or distress from the procedure. Alternatively, mild pain, suffering or distress that is long-lasting. The procedure is likely to cause moderate impairment of the well-being or general condition of the animals)
4. Severe (The animals are likely to experience severe pain, suffering or distress from the procedure. Alternatively, moderate pain, suffering or distress that is long-lasting. The procedure is likely to cause severe impairment of the well-being or general condition of the animals).

In Sweden there is a fifth category, non-recovery organ sampling, which concerns animals that have been euthanized solely for the purpose of harvesting their organs and/or tissues (Ljung & Bornestaf, 2018). Animals that are euthanized for such a reason, without any preceding procedure, are not included in the European definition of an experimental animal, unless they are genetically modified in a way that may be harmful for the animal (Ljung & Bornestaf, 2018; Directive 2010/63/EU).

1.3. Application for ethical permit

Before conducting experiments which include the use of animals you must obtain the correct permits and be adequately educated and trained in the procedures involved (Swedish Animal Welfare Act [1988:534]; Directive 2010/63/EU). One permit required is an ethical permit which needs to be approved by one of the six regional animal ethics committees in Sweden (Swedish Board of Agriculture, 2019 [Swedish: Jordbruksverket]). The applicant must supply the regional committee with information regarding the personnel involved, the aim of the experiment, potential benefits (for humans, animals or the environment), details about procedures and animals involved, and answer question regarding the 3Rs (chapter 2, 15§ in the National regulation regarding the welfare of animals used in research [SJVFS 2017:40], case number L150). Sweden also has one central committee that handles appeals and evaluates the experiments after they have been conducted (Central animal experiments ethics committee, 2019). The application for an ethical permit is considered a public document and can only be accessed and submitted in Swedish. The animal ethics committees are composed of 14 individuals, two of which are legal experts (the chairman and co-chairman) (Swedish Board of Agriculture, 2019). The remaining 12 are divided into two groups; one half consisted of scientific experts (scientists, veterinarians and animal technicians) and the other half of laypersons (local politicians and individuals from animal welfare organisations) (Ideland, 2009; Swedish Board of Agriculture, 2019).

1.4. The use of animals in research

Animals have been used in experiments for as long as humans have performed any type of research (Franco, 2013), and even though it has been common practise for such a long time it still remains a very controversial issue (Röcklinsberg *et al.*, 2017). On the one hand, people argue that the benefits (or potential benefits) that come from animal experiments outweigh the harms to the animals involved. Others argue that humans have no right what so ever to use animals for our own benefits (Röcklinsberg *et al.*, 2017). The current legislation concerning animal experiments can be seen as a mediator between these two very separate standpoints. It states that animal experiments can be performed, but only if certain standards and criteria are met. Research cannot be performed on animals without considering their welfare before, during and after the experiment (Röcklinsberg *et al.*, 2017). The World Organization for Animal Health (OIE, 2018) states that animal welfare refers to the physical and mental state of animals and how they are coping with their living conditions. Good animal welfare requires species appropriate husbandry, veterinary treatment (if necessary) and humane handling as well as humane and appropriate killing methods (OIE, 2018). One widely recognized method to minimize animal suffering, and thereby perform ethically acceptable research, is to incorporate the 3R principle in the planning of experiments (Röcklinsberg *et al.*, 2017).

1.5. The 3R principle

The 3R principle was first introduced in 1959 by Russell and Burch, in their book *The Principles of Humane Experimental Technique* (Russell & Burch, 1959; Tannenbaum & Bennett, 2015). Their aim was to improve the treatment of animals in research through the concepts of *Replacement*, *Reduction* and *Refinement* (Tannenbaum & Bennett, 2015).

*“**Replacement** means the substitution for conscious living higher animals of insentient material. **Reduction** means reduction in the numbers of animals used to obtain information of a given amount and precision. **Refinement** means any decrease in the incidence or severity of inhumane procedures applied to those animals which still have to be used.”*

– Russell & Burch, 1959

The 3R principle is no longer just an ethical question, it is also important from a research quality and better practice point of view, with better results as a positive consequence, as well as more cost-effective research (Flecknell, 2002; Clark, 2018). Utilizing the replacement principle also gives the advantage of avoiding bureaucracy, something that takes away time from designing and performing research (Baker 2016). There is, for example, no need for an ethical permit if no animals are used. The idea of the 3R principle is now widespread throughout the scientific community (Tannenbaum & Bennett, 2015) and by many considered the best approach to handle the ethical dilemmas that comes hand in hand with animal research (Franco & Olsson, 2014).

The 3R principle is regulated in EU-directive 2010/63 (article 4). According to Clark (2018) the aim of the directive is to raise standards of animal welfare across Europe and harmonise the use of animals in research between countries. The directive states that:

- 1) “Member States shall ensure that, whenever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used instead of a procedure”

- 2) “Member States shall ensure that the number of animals used in projects is reduced to a minimum without compromising the objectives of the project”
- 3) “Member States shall ensure refinement of breeding, accommodation and care, and of methods used in procedures, eliminating or reducing to the minimum any possible pain, suffering, distress or lasting harm to the animal”

The 3R principle is also incorporated in 19§ in the Swedish animal welfare act (1988:534), which concludes that animals may only be used in research if; there is no other satisfactory method, besides using animals, to reach the objectives of the study, the fewest possible number of animals are used, and if measures are taken to ensure that no animal is exposed to greater distress/suffering than what is absolutely necessary (free interpretation into English).

1.6. The creation of the Swedish 3R centre

According to EU directive 2010/63, article 47 (1), the Commission and all member states shall contribute to the development of alternative methods, i.e. methods that can lead to the same or higher levels of informational uptake, than that retained from animal experiments. All member states shall provide the nation with a point of contact which coordinates “advice on the regulatory relevance and suitability of alternative approaches proposed for validation” (article 47 [5]). All member states shall also create a national committee for the protection of animals used in research. Its purpose is to advice animal welfare bodies and authorities on matters related to the use of experimental animals (article 49 [1]).

In 2014 the Swedish government assigned the Swedish Board of Agriculture to form a centre for the 3Rs. The Swedish 3R centre has been under construction since the end of 2016 and as of the 1st of January 2018 the centre has been in active administration (Swedish Board of Agriculture 2018a). The centre is meant to act as an executive agency for Sweden’s national committee for the protection of animals used in research (Swedish Board of Agriculture, 2018b). To gather information on how to best assign their resources the Swedish 3R centre put together a survey, with the assistance of a survey company (Nordiska Undersökningsgruppen AB). The objective of this survey was to find out more about what it is that researchers in Sweden implement when it comes to the 3Rs, how they want to use the 3R centre, to identify barriers that hinders the implementation of the 3Rs and to give the Swedish board of agriculture a baseline to compare to in the future. The collected data from this survey is the basis of this master’s thesis.

1.7. Attitudes and behaviour

Measuring attitudes are difficult and predicting behaviour based on attitudes even harder (Heberlain, 2012). Attitudes and behaviours are not highly correlated and outside factors seem to have greater influence on a person’s behaviours (Heberlain, 2012). Another aspect which influences a person’s current behaviour is past behaviours, and especially the frequency of which this behaviour has been performed (Ajzen, 2001). It is also possible for an individual to have two seemingly different attitudes towards the same object, and even when we do not intend to evaluate an object or a situation, humans unconsciously have evaluative reactions (Ajzen, 2001). However difficult to assess, attitudes are often a necessary condition for behaviour, but not sufficient on its own, and attitudes can change if the person inhabiting them gain more direct experience (Heberlain, 2012).

2. Objective

2.1. Overall objective

The overall objective is to gain a deeper knowledge of Swedish researchers' attitudes to the 3Rs, find out more about what they implement when it comes to the 3Rs, and improve the conditions for future 3R efforts in Sweden.

2.2. Direct objective

The direct objective with this study is to answer the following questions:

- 1) Is there any connection between the respondents' background and their attitude to the 3Rs? (Concerning education, sector of work, experience with animal research and when they first heard about the 3Rs)
- 2) Are those researchers who have a more positive attitude towards the 3Rs also the ones who report discussing 3R questions with their co-workers more?
- 3) Are those researchers who have a more positive attitude towards the 3Rs also the ones who report thinking about the subject more often?
- 4) What are considered to be obstacles/reasons for implementing the 3Rs and is what someone mentions as obstacles/reasons connected in any way to that person's attitudes towards the 3Rs?
- 5) What are the researchers' different attitudes to the three separate parts of the 3R principle?

3. Materials and methods

3.1. The sample population

The sample population consisted of all researchers in Sweden who had applied for an ethical permit (experimenters) during the period 2016-09-01 to 2017-08-31. This group was made up by 589 unique individuals. Out of these, 572 individuals had an accessible email address which the survey could be sent to. After the survey had been sent out, six people replied that they did not work with experimental animals, two were not available during the collection period of the survey and nine resulted in an automated response that the email address was incorrect. This left 555 individuals that could potentially answer the survey. The survey was emailed to the sample group on the 22nd of November 2017, with reminders sent out on the 27th and 30th of November. The collection period ended on the 4th of December and by then 215 people had answered (39 %). The survey was available in both Swedish and English and out of the 215 replies only 32 were in English.

3.2. Literature

The literature for this study was collected from the databases *Web of Science* and *Google Scholar*. The search words that were used consisted of: "3R", "Animal", "Research", "Welfare", "Ethics", "Attitudes" and "Experiments" (used in different combinations). After

using the search words the first ten pages (with ten articles per page) were scanned for articles with a relevant title. The abstract of articles selected, based on this system, were read and if something relevant was found the article was saved, all others were discarded. Further articles were found in the reference list of other articles and some were received from the supervisors and others connected to this project.

3.3. Determining attitudes

Two questions in the survey were chosen to represent if the respondents had a more positive or negative attitude towards the 3Rs. Those questions were:

1. The extensive focus on the welfare of the animals will hinder scientific breakthroughs
2. Further implementation of the 3Rs will be detrimental to the quality of my results

The choices they had were the following:

- Strongly disagree (1)
- Disagree (2)
- Neither agrees nor disagrees (3)
- Agree (4)
- Strongly agree (5)

Those respondents that filled in “Strongly disagree” or “Disagree” (1 or 2) were considered to have a positive attitude towards the 3Rs. Those who filled in “Agree” or “Strongly agree” (4 or 5) were considered to have a negative attitude towards the 3Rs.

3.4. Data analysis

The data from the survey were organized in Excel (2007) and thereafter transferred into the program R (a language and environment for statistical computing) (R Core Team, 2018). The individuals that had answered “1 or 2” or “4 or 5” were filtered out, into separate data sets, and the individuals that had answered “1 or 2” or “4 or 5” in both questions were also gathered into separate data sets. This resulted in the following data sets:

1. The complete data set
2. PosWelfare = Individuals who answered 1 or 2 in question 1
3. NegWelfare = Individuals who answered 4 or 5 in question 1
4. Pos3R = Individuals who answered 1 or 2 in question 2
5. Neg3R = Individuals who answered 4 or 5 in question 2
6. PosWelfare3R = Individuals who answered 1 or 2 in both questions
7. NegWelfare3R = Individuals who answered 4 or 5 in both questions

To find out what the respondents in the six different subgroups had answered, in the eight questions that were selected (Annex 1), barplots were created. To see if there were any significant differences between the answers of the different subgroups a Kruskal-Wallis test was used. The positive groups were compared to the negative groups in the following manner; PosWelfare vs. NegWelfare, Pos3R vs. Neg3R and PosWelfare3R vs. NegWelfare3R. For some questions there were no test for significance performed, the analysis of these will be purely descriptive.

4. Results

4.1. Sample characterization

The survey was answered by 100 women (46 %), 107 men (50 %) and 8 individuals that did not want to state their gender (4 %). The dominating age group (47 %) were between 45 and 64 years old. However, a large part of the respondents were also represented in the 35-44 years of age category (34 %). There were no individuals under the age of 25 in this survey; only 6 % between the ages of 25-34 and 6 % were 65 or older. In total, 7 % of the respondents did not want to state their age.

A majority of the respondents had a background in biology (55 %). Medical doctors accounted for 18 %, chemists and pharmacists 7 % and veterinarians 7 %. A total of 13 % stated that they had a different educational background to the ones suggested in the survey. Represented here were individuals educated in biomedicine, ecology, dentistry, and veterinary nursing, amongst others.

Respondents working either at a university or at a county council were in majority in the data set (83 %). Only 8 % worked within the pharmaceutical industry and 6 % worked at other companies or other authorities. A low 3 % stated that they worked with something else. They were, however, not able to declare where they worked, in this survey.

Regarding the respondents' experience of working with experimental animals 31 % stated that they had more than 20 years of experience, 28 % had more than 10 years of experience and 19 % had up to 10 years. A total of 22 % chose not to answer this question.

4.2. Identifying individuals with positive or negative attitudes

Number of individuals that chose 1 or 2 (positively inclined to the 3Rs):

- Question 1 = 85 individuals (PosWelfare)
- Question 2 = 84 individuals (Pos3R)
- Question 1 and 2 = 57 individuals (PosWelfare3R)

Number of individuals that chose 4 or 5 (negatively inclined to the 3Rs):

- Question 1 = 68 individuals (NegWelfare)
- Question 2 = 68 individuals (Neg3R)
- Question 1 and 2 = 38 individuals (NegWelfare3R)

4.3. Background of the respondents in the six subgroups

Regarding educational background biologists made out the majority in all six subgroups. This was expected since they also were in majority in the complete data set. There were no statistically significant differences found between any of the groups for this question with the Kruskal-Wallis test. However, worth noticing is that there were no chemists represented neither in Neg3R nor NegWelfare3R and there were no veterinarians in NegWelfare3R.

Same as for the complete data set, university or county council employees made up the majority of all subgroups. There were no statistically significant differences found between the positive and negative groups for sector of employment. Based on the figures alone, there were a higher percentage of the negative groups that worked at a university or at a county council, compared to the positive groups (Fig. 1.). The opposite could be seen for

individuals working within the pharmaceutical industry, where there were a higher percentage part of the positive groups. Worth noticing here is that, apart from 1 % from Neg3R, there were no individuals from the negative groups that worked for any other company or at any other authorities, and that all individuals (100 %) in NegWelfare3R worked at a university or at a county council.

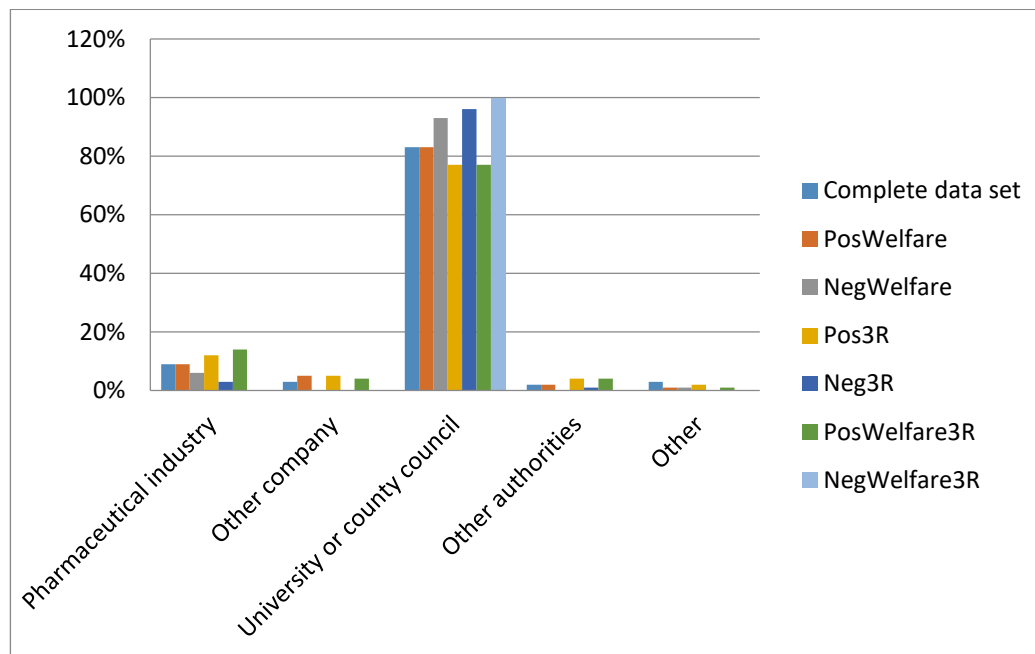


Figure 1. Answers to the question regarding sector of employment for all seven data sets.

A majority of the individuals in all subgroups had more than 10 years of experience working with animal research (Fig. 2). The largest difference within a group could be seen in NegWelfare3R where 89 % had more than 10 years of experience and only 11 % had 10 years or less. There was a statistically significant difference found between PosWelfare and NegWelfare ($P = 0.01611$) and between PosWelfare3R and NegWelfare3R ($P = 0.01835$) regarding the individuals experience with animal research. In this case the negative groups had a higher percentage of the respondents with more than 10 years of experience (PosWelfare 74 % vs. NegWelfare 86 %, PosWelfare3R 77 % vs. NegWelfare3R 89 %). There was no statistically significant difference found between Pos3R and Neg3R with the Kruskal-Wallis test.

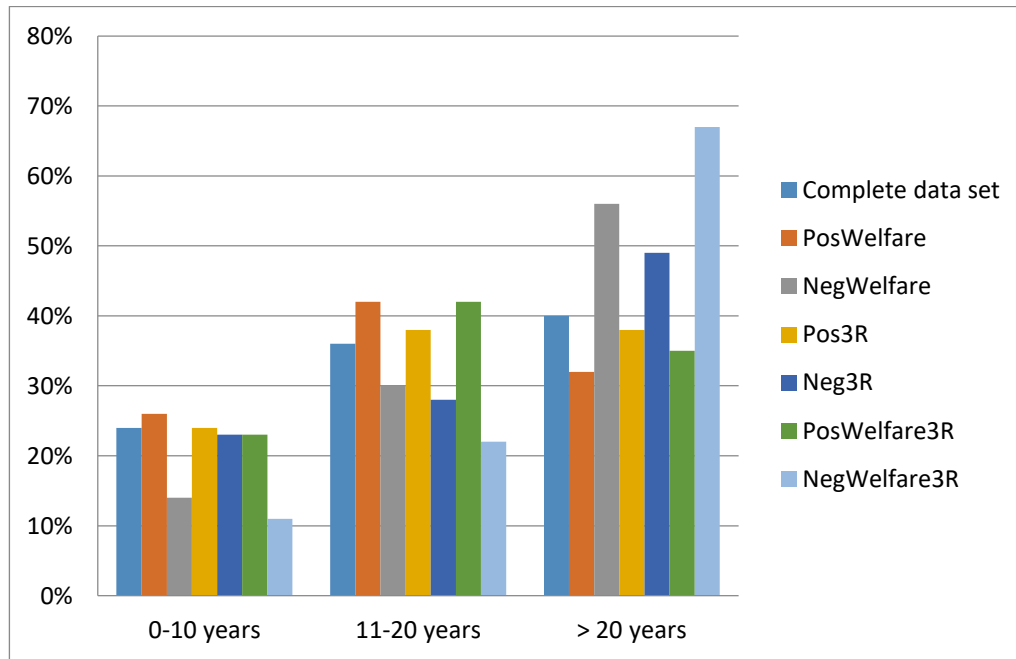


Figure 2. Years of experience working with animal experiments for the complete data set and for the six subgroups.

A total of 22 % of all the respondents in the survey chose not to answer the question about experience. For the six subgroups, the response rate for this question was generally higher for the positive groups compared to the negative groups (Fig. 3).

Figure 3. The response rate for the six subgroups regarding experience with animal experiments.

Data set	Response rate	Data set	Response rate
PosWelfare	85 %	NegWelfare	74 %
Pos3R	86 %	Neg3R	78 %
PosWelfare3R	84 %	NegWelfare3R	71 %

Only a small number of all the individuals (5 %) had never heard of the 3Rs until they took this survey. For NegWelfare and NegWelfare3R approximately half of the individuals had heard about the 3Rs less than 10 years ago and the other half had heard about it more than 10 years ago. In all the other groups the majority of the individuals had heard about the concept of the 3Rs more than 10 years ago (Fig. 4). However, there were no statistically significant differences found between the positive and negative groups with the Kruskal-Wallis test.

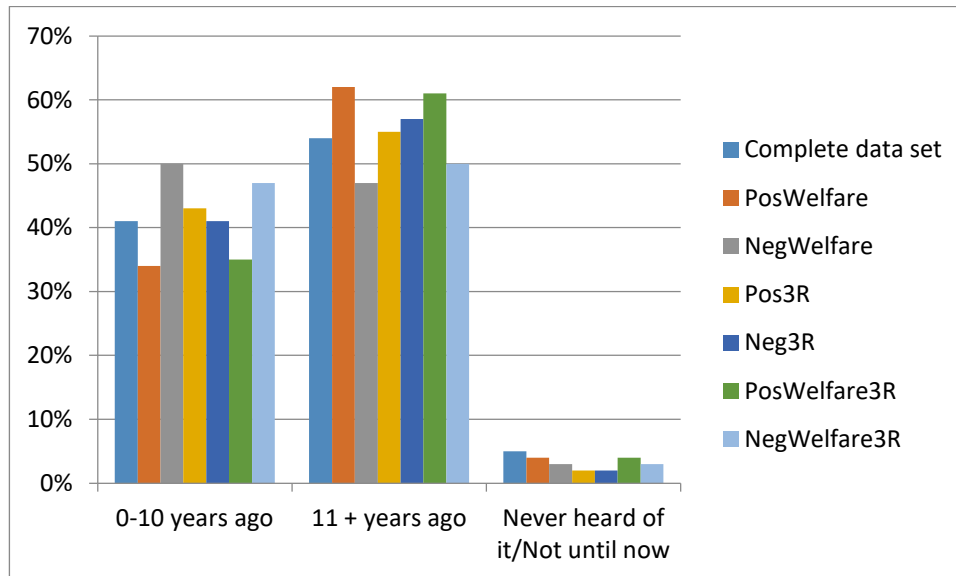


Figure 4. The distribution of when the respondents had heard about the 3R principle for the first time.

4.4. How often do the respondents discuss and think about the 3Rs

Regarding how often the respondents discuss 3R questions with their co-workers, there were no statistically significant differences found between any of the subgroups for any of the three questions (Annex I). However, there are some indications that refinement and reduction is being discussed more frequently than replacement. For refinement, 57 % of all the respondents claim to discuss the subject frequently or very frequently. For reduction this figure is 54 % and for replacement it drops to 40 % (Fig. 5).

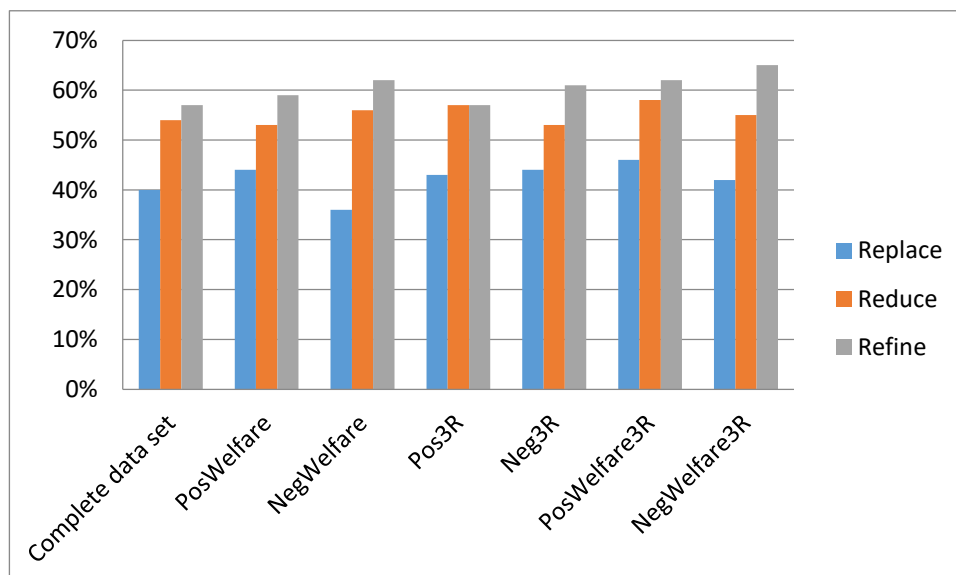


Figure 5. Respondents that claim to discuss the 3Rs with their colleagues frequently or very frequently.

The majority of the respondents foremost think about the 3Rs when they are applying for an ethical permit (Fig. 6). This was also true for all six subgroups. Regarding the options

“When coming into direct physical contact with animals” and “When attending seminars, conferences and other meetings” there were very little difference between the seven groups (percentages ranging between 47 to 55 % and from 21 to 26 %, respectively). For the options “When applying for research funding” and “When applying for an ethical approval at the regional ethics committee” there were a slightly higher percentage of the negative groups that chose these options compared to the positive groups. The largest difference could be seen between PosWelfare3R (39 %) and NegWelfare3R (58 %) for the option regarding research funding and between PosWelfare (69 %) and NegWelfare (87 %) for the option regarding the ethical permit. For the option “All the time as part of my day-to-day work” the opposite could be seen, were the positive groups had a higher percentage than the negative groups. The largest difference there could be seen between PosWelfare (58 %) and NegWelfare (43 %). Concerning the option “Other” there were few individual that chose this option, for all seven groups (percentages ranging between 6 and 12 %).

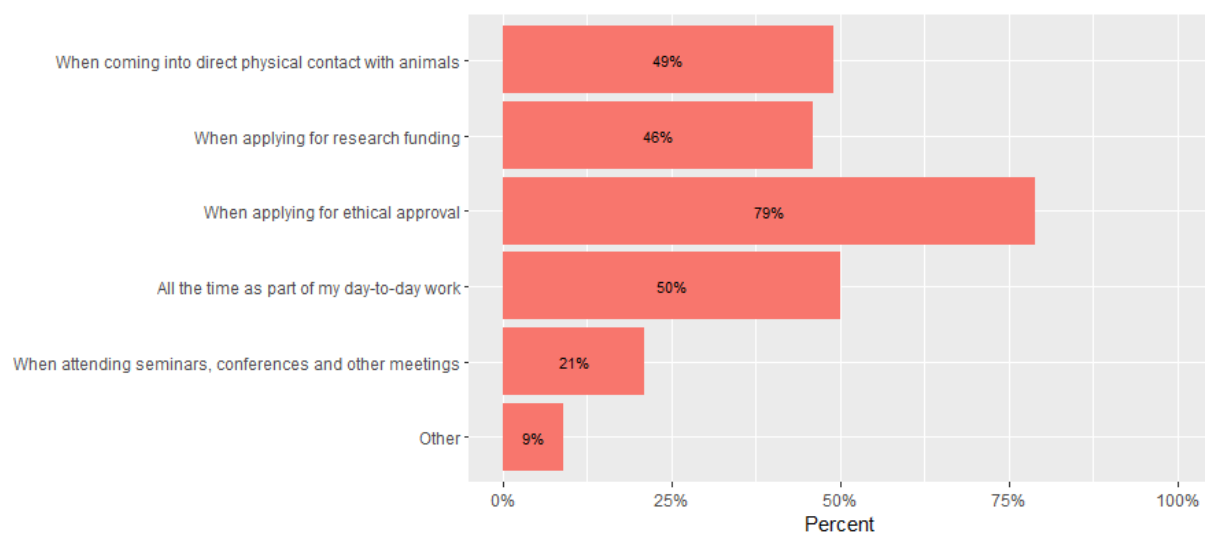


Figure 6. Description of when the respondents consider the 3Rs in their work (complete data set).

4.5. Obstacles and reasons for implementation of the 3Rs

For all the respondents in the survey, the top three obstacles for implementing replacement were as follows:

1. Comparability of data (50 %)
2. Lack of appropriate scientific or technological innovation (44 %)
3. Other obstacle (33 %)

The following comments were selected to represent some of the suggestions for “Other obstacles”, for this question:

(The original Swedish sentence in brackets, free interpretation into English)

- Certain scientific questions need animal experimentation (Answer in English)
- We work with research concerning animal welfare where animals often are necessary unless we work with questionnaires etc. (Vi arbetar med forskning kring djurvälstånd så där är djuren ofta nödvändiga om vi inte arbetar med enkäter etc)
- An impossibility with electrical fishing (Omöjligt vid elfiske)
- I work with wild birds, there is no other method for the questions we work with (Jobbar med vilda fåglar, finns ingen annan metod för de frågor vi arbetar med)
- Difficulty in getting research published using alternative methods (Answer in English)

The same three options came in on top three for all the six different subgroups as well. They were, however, not in the same order for all the groups (Fig. 7).

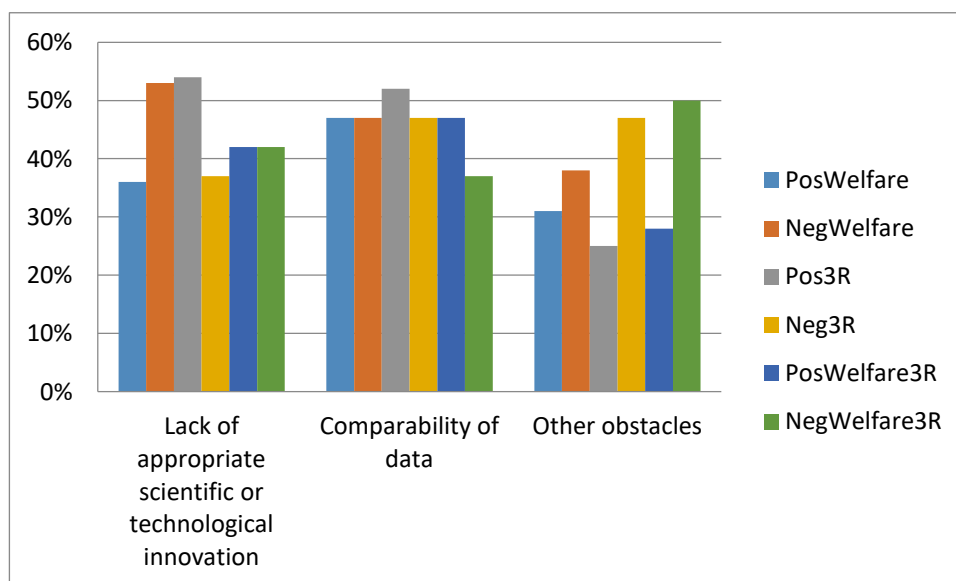


Figure 7. The top three obstacles for replacing animals in experiments with other methods, for the six subgroups.

The option “*Lack of time due to other duties*” was generally not considered a hindrance for implementation of replacement, only between 4 and 7 % of the respondents in the different groups chose this option. Neither was funding considered an issue as only 5 to 12 % chose this option. Regarding the option “*Legislation or regulatory opportunities*” there were a larger difference between the positive and negative groups, with the positive groups choosing this option more than the negative groups. The largest difference could be seen between PosWelfare3R (23 %) and NegWelfare3R (5 %).

For implementing reduction, the top three obstacles for all the respondents in the survey were as follows:

1. Comparability of data (40 %)
2. Other obstacle (28 %)
3. Lack of appropriate scientific or technological innovation (27 %)

The following comments were selected to represent some of the suggestions for “*Other obstacles*” for this question:

(The original Swedish sentence in brackets, free interpretation into English)

- Statistical power (Statistisk säkerhet)
- Number of caught fish cannot be influenced (Antalet fångade fiskar går inte att påverka)
- A relevant number of animals is required for conclusive results (Ett relevant antal djur krävs för att få konklusiva resultat)
- The focus on reducing the number of animals could potentially worsen the situation for the animals that is part of the study. This must be changed in L150 to make it possible (Fokus på mindre antal djur kan till viss försämra situationen för de djur som ingår i studier. Detta måste ändras i L150 så att det är möjligt)

“Comparability of data” came in 1st place for all the subsets, apart from NegWelfare3R, where it came in shared 1st place with “Other obstacle” (Fig. 8). The options “Lack of time due to other duties”, “Insufficient funding available” and “Legislation or regulatory opportunities” were not considered as obstacles for implementation of reduction. Only a small proportion of the respondents filled in this option (< 10 %, for the complete data set).

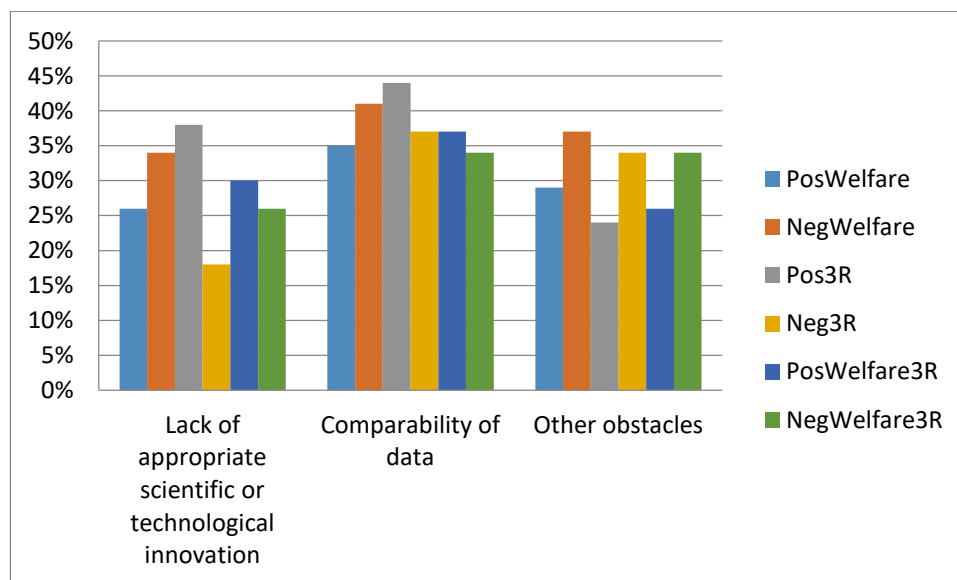


Figure 8. The top three obstacles for reducing the number of animals in experiments, for the six subgroups.

For all the respondents in the survey, the top three obstacles for implementing refinement were as follows:

1. Lack of appropriate scientific or technological innovation (21 %)
2. Insufficient funding available (18 %)
3. Comparability of data (11 %)

The option “Other obstacle” came close with 10 %, so the following comments were selected to represent some of the suggestions for “Other obstacles” for this question:

(The original Swedish sentence in brackets, free interpretation into English)

- Animal Rights Sweden that want to make it harder for animal research not improve them (Djurens rätt som vill försvåra djurförsök inte förbättra dem)
- Difficult to balance against the purpose of some experiments (Svårt att balansera mot syftet med vissa försök)
- We already handle them very well, cannot see how we can improve handling (Vi hanterar dem redan väldigt väl, kan inte se hur vi kan förbättra hanteringen)
- We need to maintain the competence e.g. hire more researchers that are trained in working with animal research (Vi behöver behålla kompetensen dvs anställa fler forskare som har gedigen träning att arbeta med djurförsök)

NegWelfare, Pos3R, Neg3R and NegWelfare3R all had “Lack of appropriate scientific or technological innovation” as their number one obstacle for implementation of refinement (Fig. 9). PosWelfare and PosWelfare3R both had “Insufficient funding available” in first place. The options “Lack of time due to other duties” and “Legislation or regulatory opportunities” were only chosen by less than 10 % of the individuals in all of the subgroups.

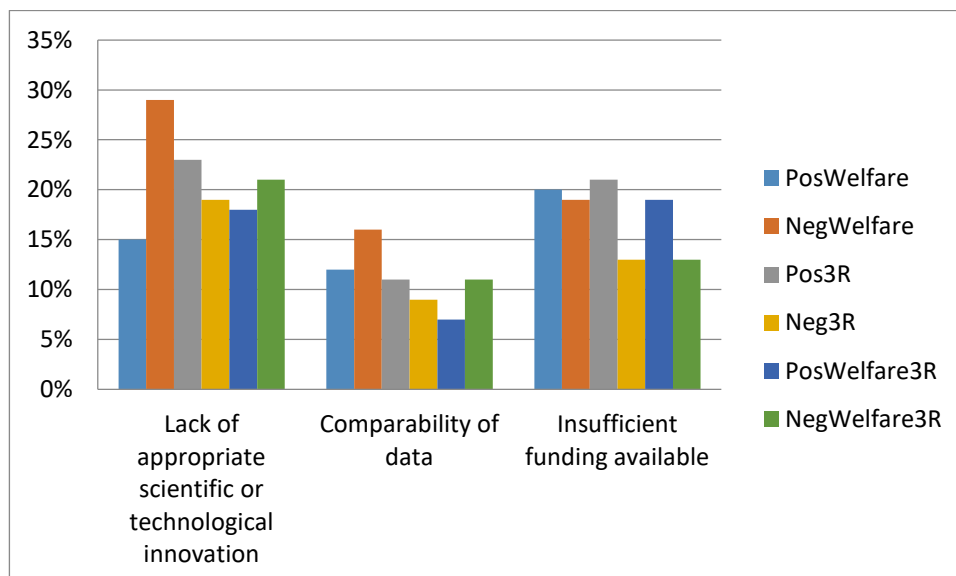


Figure 9. The top three obstacles for refining the welfare of animals in experiments, for the six subgroups.

For the complete data set the top three reasons for implementing the 3Rs were as follows:

1. Ethical reasons (89 %)
2. Quality reasons (41 %)
3. Economic reasons (25 %)

The top three reasons for implementation were the same for all six subgroups as for the complete data set (fig. 10). A slightly higher percentage of the positive groups chose the options “*Ethical reasons*” and “*Quality reasons*” more than the negative groups. Out of all the respondents only 1 % thought that there were no reasons for implementing the 3Rs.

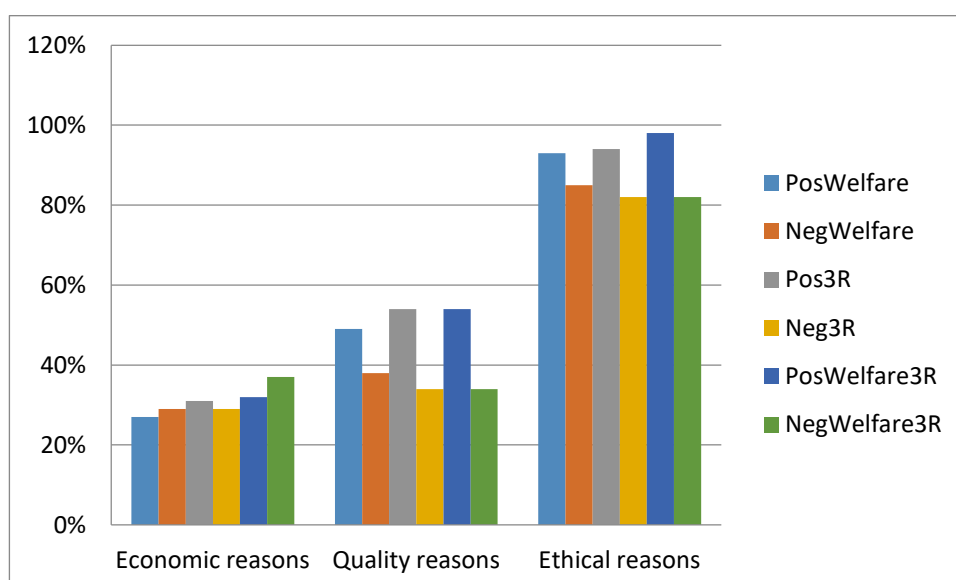


Figure 10. The top three reasons for implementation of the 3Rs according to the six subgroups.

In total, 50 % of all the respondents think that there are no obstacles when it comes to refining procedures, experiments and environment. That figure drops to 17 % for reducing

the number of animals in experiments and to a low 6 % for replacing animal experiments entirely. The same trends can be seen in the six different subsets of the data (Fig. 11). There were some indications that the positive groups chose the option (“*There are no obstacles*”) more than the negative groups, when it comes to refinement. The largest difference could be seen between the individuals in PosWelfare3R and NegWelfare3R (60% and 42% respectively).

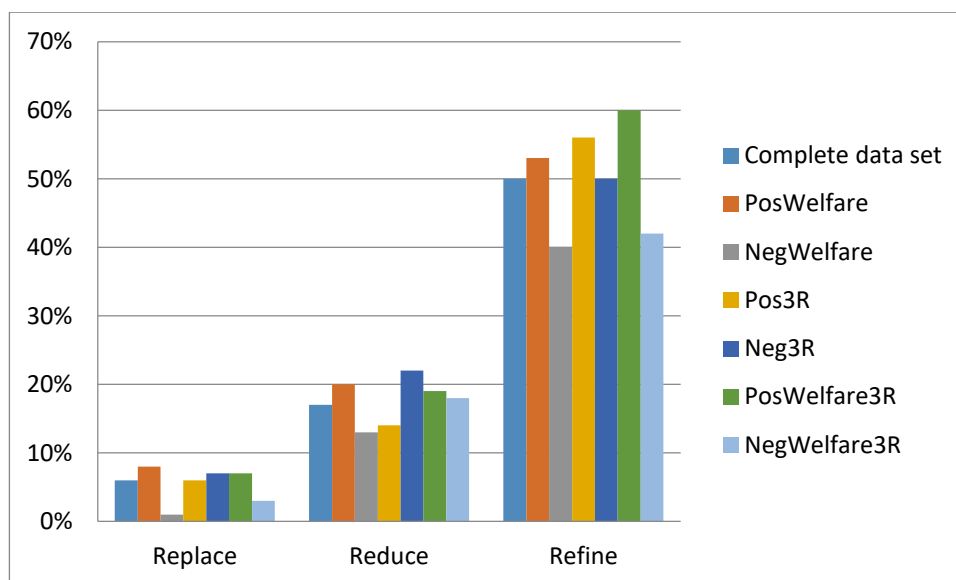


Figure 11. Percentage of individuals that chose the option “there are no obstacles” for the question “which do you consider to be the main obstacles to implementing the 3Rs when it comes to; replace animal experiments with other methods, reduce the number of animals used in procedures and refine the welfare of animals in procedures”.

5. Discussion

5.1. Attitudes in relation to the respondents’ background

There was a very even distribution between men and women in this survey and a reasonable representation of the different age categories, considering that individuals under the age of 25 rarely have graduated from university and that individuals over the age of 65 might have retired from work. The first question focused on the respondent’s background in relation to their attitudes towards the 3Rs. There were no statistically significant differences between the positive and negative groups concerning educational background, sector of employment and when they first heard of the 3R principle. A difference was, however, found regarding the respondents experience with animal research for two of the three positive/negative pairs (Pos/NegWelfare and Pos/NegWelfare3R). A higher percentage of the individuals that were classified as having a more negative attitude towards the 3Rs had more than 10 years of experience, compared to the ones with a more positive attitude (PosWelfare 74 % vs. NegWelfare 86 %, PosWelfare3R 77 % vs. NegWelfare3R 89 %). Nevertheless, the majority of the two positive groups also had more than 10 years of experience. The cause of this difference and possible consequences needs to be further investigated before any conclusions can be drawn.

Even though the majority of the respondents (76 %) had more than 10 years of experience of working with animal research, only 55 % reported that they had first heard about the 3Rs more than 10 years ago. One can only speculate about why there is a difference in these figures but it leads to the thought that some individuals started working with animal research but were not introduced to the concept of the 3Rs until later. According to Tannenbaum & Bennett (2015) the 3R principles did not gain widespread recognition when the book was first published (in 1959) and it was not until several years later that the ideas of Russell and Burch spread through the scientific community. In connection with its recognition, a special edition version of the book, containing the original text, was reissued in 1992. Therefore, according to the article by Tannenbaum & Bennett (2015), the 3R principle has been widely known by scientists for at least 27 years. The results showed that 86 % of NegWelfare individuals had more than 10 years of experience with animal research but only 47 % had heard about 3R more than 10 years ago, compared to PosWelfare where 74 % had more than 10 years experience and 62 % had heard about the 3Rs more than 10 years ago. Why there is a difference in percentages between the positive and negative groups needs to be further investigated.

A positive finding, from a 3R perspective, was that only a small percentage of all the individuals in this study had never heard of the 3Rs until they received the survey. It was not in the scope of this master thesis to examine how many years of experience these individuals have with animal research; it is, however, possible to examine this with the existing data. Nevertheless, since the 3Rs is part of the current legislation concerning experimental animals it is difficult to believe that individuals with great experience of working with animal research would have missed the concept of the 3Rs. According to chapter 6, 13§, Table 1, in the National regulation regarding the welfare of animals used in research (SJVFS 2017:40), case number L150, all who perform research on animals, design such experiments, care for and/or euthanize animals used in research needs to be educated in the ethics surrounding animal research, animal welfare and the 3R principle.

In comparison, a study performed by Franco *et al.* (2018) showed that 42 % of the respondents in their survey did not know of the 3R principles and almost 5 % claimed to know about them but could not recite them correctly. Their survey was answered by individuals (mostly from Europe) attending courses in laboratory animal science (LAS) held in Denmark, Germany, Portugal and Switzerland. One possible explanation for this difference could be that the respondents to the survey performed in Sweden were all experimenters, individuals that design the experiments. One can assume that they would already have taken part in a LAS course (or similar), since this is compulsory for anyone wanting to work with experimental animals (Directive 2010/63/EU; Swedish animal welfare act [1988:534]). This might also be an explanation to why none of the respondents to a survey performed in Denmark (Nøhr *et al.*, 2016) reported that they did not know of the 3Rs at all and only 8 % reported that they did not know of them very well. The Danish survey was emailed to individuals holding a license to perform animal experiments in Denmark, and they were then asked to forward the survey to researcher within their organization.

5.2. When and how the respondents discuss and think about the 3Rs

The second and third question focused on when the researchers discuss and think about the 3Rs in their work. The results showed that the respondents foremost think about the 3Rs when they are applying for an ethical permit. Interestingly, only 79 % filled in this option and since the 3Rs are a large part of the application form for an ethical permit these results

were surprising. However, the option “*All the time as part of my day-to-day work*” may have affected the response rate for the option about ethical approval. Some individuals might have filled in only this option, as it covers all of the other answers. There were some indications that the positive groups chose the option “*All the time as part of my day-to-day work*” more than the negative groups did. At the same time the negative groups chose the options “*When applying for research funding*” and “*When applying for an ethical approval*” more than the positive groups. This may indicate that if you have a more negative attitude towards the 3Rs you think about the subject when you are in situations where you have to, but they are not present in your mind throughout your daily work.

No difference was found between the positive and negative groups regarding how often they discuss the 3Rs with their colleagues, but the differences in percentages indicate that the respondents discuss the separate parts of the 3Rs to a different amount. Refinement is being discussed most, followed by reduction and lastly replacement. It is difficult to draw any conclusions regarding the researchers’ attitudes towards the separate parts of the 3Rs (question five) from these results. However, considering that half of the respondents do not see any obstacles when it comes to refinement, only 17 % think the same for reduction and a low 6 % for replacement it might be possible to say that the respondents see refinement as less problematic compared to reduction and replacement. A survey performed on researchers in the United Kingdom in 2008 by the National Centre for Replacement, Refinement and Reduction of Animals in Research (NC3R, 2008) showed that 73 % of the respondents agree with the statement that “complete replacement of the use of animals in research and testing will never be achieved”. A possible explanation for why the researchers discuss replacement less might be because of a similar belief as the researchers in the UK. Franco *et al.* (2018) drew the conclusions from their survey that the current prioritization order for the 3Rs were; Refinement, Reduction and Replacement. This is a complete reversal of the order proposed by Russell and Burch in 1959 (Russell & Burch, 1959). The results from this survey might indicate that Swedish researchers prioritize the three parts of the 3R principle in the same manner.

5.3. Obstacles and reasons for implementation

The fourth question focused on what the Swedish researchers considered obstacles/reasons for implementation of the 3Rs. The main obstacles for implementation that came up corresponds well with the results found in the Danish survey (Nøhr *et al.*, 2016), where the top two obstacles were lack of appropriate scientific or technological innovation and comparability of data. Concerning replacement, the same options that came in top three for all the respondents also came in top three for all the six subgroups. They did differ a bit regarding the order, which option that came in which place, but the differences were small and there were no pattern to it. There were some indications suggesting that the positive groups thought that legislation and other regulations were more of a hindrance for implementation of replacement than the negative groups did (PosWelfare 18 % vs. NegWelfare 3 %, Pos3R 17 % vs. Neg3R 6 % and PosWelfare3R 23 % vs. NegWelfare3R 5 %). If this means that they would like to be able to do more concerning replacement need to be further examined. Ethical reasons were considered by the respondents to be the foremost reason for implementing the 3Rs. This was followed by quality and economic reasons, which is in line with the articles by Flecknell (2002) and Clark (2018), mentioned in the introduction.

Nature published a news article in 2015 regarding improving statistical power of animal studies. In this article, Cressey (2015) stated that funders in the United Kingdom are putting stronger pressure on scientists to prove beforehand that their experiments are using enough animals to get statistically valid results. If this is not proven beforehand the applicant may risk rejection of their application (Cressey, 2015). There is an ethical conundrum with reduction, on the one hand you want to use as few animals as possible, but on the other hand you cannot use too few animals or the experiment will not result in statistically useful results. According to Cressey (2015) the funders' aim with tightening the guidelines was to eliminate studies where the animals groups are too small (resulting in statistically invalid results), and thereby avoid wasting animal lives in vain. This of course puts higher pressure on researchers' knowledge about statistics and means that they cannot rely on what has been done historically, regarding sample sizes. In this survey half of the comments concerning obstacles for reducing the number of animals in experiments mention statistical power. It is important to remember the meaning of reduction and what it aims to eliminate. Simply reducing the number of animals to the smallest number possible, without considering if it will result in statistically valid results, is not in compliance with the 3Rs. By performing statistical test to determine how large sample sized an experiment needs to achieve valid results the researchers are already complying with the reduction part of the 3Rs.

5.4. Evaluating the survey

Based on the comments in the comment sections not all respondents felt that the survey was suited to them and their scientific work. In comparison with the Danish survey (Nøhr *et al.*, 2016) this study included more individuals that reported having a different educational background to the ones suggested (13 % in Sweden, 3 % in Denmark). Most of the remarks concerning this came from people working with wild living animals. Nevertheless, according to chapter 6, 12§ in the National regulation regarding the welfare of animals used in research (SJVFS 2017:40), case number L150, those who catch free living wild animals for research needs to be educated in minimizing stress and suffering for the animals. With this in mind, it is understandable that the Swedish 3R centre wanted to create a survey that included and yielded as much information as possible. However, it might have been beneficial to target the different areas of research separately (e.g. laboratory, agriculture, wild life, pets etc.).

The translation "*Legislation or regulatory opportunities*" may have caused problems for the English speaking respondents. In Swedish it says "Lagstadgade krav eller andra regler", which can be translated to "Statutory requirements or other rules". The Danish survey wrote "Legislation or regulatory requirements", for this option. The meaning of this option might have been lost in translation for some of the 32 individuals that replied to the English version of the survey.

It is also important to consider the individuals that answered the survey. Was it only individuals interested in the 3Rs (positively or negatively) who felt inclined to answer? Are you more likely to answer a survey like this if you are interested in improving the welfare of animals, or if you are feeling that it hinders your research? Only 39 % of the original population (the ones who had applied for an ethical permit during the chosen time period) answered the survey, the reason for this is difficult to find out but the relatively short reply period might have something to do with it. The survey was only available for 13 day, out of which 4 were non working days (weekend). The sample sizes for each of the subgroups were

also small; PosWelfare 85, NegWelfare 68, Pos3R 84, Neg3R 68, PosWelfare3R 57 and NegWelfare3R 38. This might have had an effect on the results in this study.

The results from this survey and the comments in the comment sections raise the question about the researchers' definition of the 3Rs. Is it possible to assume that all 215 individuals apply the same definition of the 3R principle, or even the same definition of the three separate parts of it? In the survey performed in the UK (NC3R, 2008), 29 % of the respondents chose the option "Replacing 'higher' mammals with 'lower' mammals" in the question "Which of the following definition fits your understanding of replacement". In the same question for reduction, 24 % chose the option "Reducing the degree of pain and suffering caused to animals by your procedures", and for replacement 52 % chose "Improving experiments to yield better data". These questions were answered by 1529 researchers and it shows that there are some misconceptions regarding the definition of the 3Rs. Perhaps it would be beneficial to ask similar questions to researchers in Sweden.

As a complement to this survey it would be interesting to perform interviews with researchers working in Sweden. This would hopefully help with getting a clearer picture of what future 3R efforts should include and bring answers to some of the questions that were raised as a result of this survey. It would also be interesting to conduct a similar survey aimed at the animal technicians, since these are the people who handle the animals on a daily basis and in many cases implements refinement.

6. Conclusion

This study highlights the complexity surrounding researchers' attitudes to the 3Rs. It could not find any clear connection between attitude and background, nor for attitude in connection to what are considered obstacles and reasons for implementation. It did however, pinpoint areas which are considered to be obstacles for implementation and indicated that refinement might be seen as less problematic than reduction and, especially, replacement. It might therefore be useful for the Swedish 3R centre to focus on replacement, to show its possibilities and prove that it deserves its place as the number one priority of the 3Rs, as suggested by Russell and Burch. Further studies are needed to fully grasp the Swedish researcher's views of the 3Rs. Nevertheless, this study has hopefully helped with improving the conditions for future 3R efforts in Sweden.

7. Popular scientific summary

The use of animals in research has a very long and grim history and today millions of animals are used in the name of science every year. Some argue that the benefits that come from this outweigh the pain and suffering that is caused to the animals. Others argue that we have no right to use animals in this way. These are two very separate standpoints and somewhere in between we find legislation. It states that it is allowed to use animals in research, but only if certain criteria are met first. To be granted permission to perform experiment on animals both the individuals involved and the location for the experiments must be certified for such work. One widely recognized method for ensuring that the animals are treated well and that the pain and suffering is kept to a minimum is to include the 3R principle into the planning and performing of experiments. The 3Rs stands for

replace, reduce and refine and means that you have to replace animal experiments with methods not involving animals, whenever it is possible. If no such methods are available you have to reduce the number of animals used as much as possible, without it having a negative impact on the results of the study. You also have to think about the welfare of the animals that you end up using. You need to improve and refine the housing, care, procedures and other things that have an impact on the well-being of the animals.

To improve on the work with the 3Rs in Sweden the Swedish board of Agriculture created a centre for 3R. The centre wanted to find out more about the current status of the 3Rs in Sweden and designed a survey aimed at researchers who are part of planning and performing animal research. The survey was the basis of this master's thesis and the aim of the study was to find out more about Swedish researchers' attitudes and views of the 3Rs. The study could not find any clear connection between the researchers' background and their attitudes to the 3Rs. Neither could it find any connection between attitude and what someone mentions as an obstacle or reason for applying the 3Rs to their work. The results did, however, point in the direction that ways of improving the conditions for the animals in experiments is being discussed the most and replacing animals with non-animal methods the least. It also showed that half of the researchers do not see any obstacles for applying refinement to their work, while only 6 % feel the same for replacement. Ethical reasons were considered to be the main reason for applying the 3Rs to animal research, followed by quality reasons and economic reasons. The possibility of comparing results with previous data and a lack of scientific and technological innovation were considered to be the main obstacles for the 3Rs. The 3Rs have the possibility of having a great impact on the lives of animals used in experiments, therefore the work needs to continue and further studies are needed to find out more about researchers views and attitudes to the 3Rs.

Acknowledgements

I would like to thank my supervisor, Lotta Berg, and my assistant supervisors, Elin Weber and Per Ljung, for their help and professional feedback on this master's thesis. Thank you also to Elin Spangenberg for being my examiner and to Phimphanit Choklikitumnuey for being my opponent. I would also like to thank Staffan Betnér for his statistical advice and Helena Röcklinsberg for her advice on ethics. Lastly, I would like to thank my fiancé Anders Pistol and our daughter Klara, for their love and support.

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Annex I

<p>When did you first hear about 3R?</p> <ul style="list-style-type: none"> ○ More than 20 years ago ○ 11-20 years ago ○ 5-10 years ago ○ 1-4 years ago ○ Within the last year ○ Never heard of 3R/not until now 	<p>Educational background</p> <ul style="list-style-type: none"> ○ Biology ○ Chemistry ○ Pharmacy ○ Medical doctor ○ Veterinarian ○ Other
<p>Employment sector</p> <ul style="list-style-type: none"> ○ Pharmaceutical industry ○ Other company ○ University or county council ○ Other authorities ○ Other 	<p>For how long have you been working with animal experiments?</p> <ul style="list-style-type: none"> ○ More than 20 years ○ 11-20 years ○ 5-10 years ○ 1-4 years ○ Less than one year
<p>When do you consider 3Rs in your work?</p> <ul style="list-style-type: none"> ○ When coming into direct physical contact with animals ○ When applying for research funding ○ When applying for ethical approval at the regional ethics committee ○ All the time as part of my day-to-day work ○ When attending seminars, conferences and other meetings ○ Other 	<p>How often do you discuss the following topics with colleagues?</p> <ul style="list-style-type: none"> ❖ Ways to increase the welfare of animals in procedures ❖ Methods to reduce the number of animals used in procedures ❖ Methods that do not require the use of animals ○ Very frequently ○ Frequently ○ Sometimes ○ Rarely ○ Never
<p>Which do you consider to be the main obstacles to implementing the 3Rs when it comes to:</p> <ul style="list-style-type: none"> ❖ Replace animal experiments with other methods ❖ Reduce the number of animals used in procedures ❖ Refine the welfare of animals in procedures ○ Lack of appropriate scientific or technological innovation ○ Lack of time due to other duties ○ Insufficient funding available ○ Legislation or regulatory opportunities ○ Other obstacle ○ There are no obstacles 	<p>Which do you consider to be the main reasons to implementing the 3R?</p> <ul style="list-style-type: none"> ○ Economic reasons ○ Quality reasons ○ Ethical reasons ○ Requirements from the management at the workplace ○ Legislative requirements ○ Requirements from funders ○ Requirements from scientific journals ○ Other reasons ○ No reasons