

Lindsjö et al.:

The 3Rs in Animal Welfare Bodies at Swedish Universities – Knowledge, Attitudes, Implementation

Supplementary Data

Appendix 1 - Questionnaire

I. Understanding of and attitude to the 3Rs

1. Which of the following definitions fits your understanding of **REPLACEMENT**?

- a. Replacing animals with in vitro techniques
- b. Redesigning experiments in order to avoid the use of animals
- c. Replacing vertebrates with invertebrates
- d. Replacing “higher” (more sentient) mammals with “lower” (less sentient) mammals
- e. Replacing animals with computer modelling techniques
- f. I don't know

Response: Tick all that apply

2. Which of the following definitions fits your understanding of **REDUCTION**?

- a. Obtaining comparable levels of information in your experiment while using fewer animals
- b. Reducing the degree of pain and suffering caused to animals by an experimental procedure
- c. Reducing the number of animals used per experiment
- d. Obtaining more information in an experiment while using the same number of animals
- e. Reducing the total number of animals used in research overall in Sweden
- f. I don't know

Response: Tick all that apply

3. Which of the following definitions fits your understanding of **REFINEMENT**?

- a. Improving experiments so that fewer animals are used
- b. Improving procedures so that the animals experience less pain and suffering
- c. Improving animal welfare by minimizing suffering and providing better housing conditions
- d. Improving the conditions in which animals are kept
- e. Improving experiments to yield better data
- f. I don't know

Response: Tick all that apply

4. To what extent do you agree with the following statements?

Scale:

Don't know	Strongly disagree	Disagree	Neither disagree/agree	Agree	Strongly agree
0	1	2	3	4	5

The 3Rs are useful during the following situations and research stages:

- a. Animal housing and environment
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ALTEX 38(3), SUPPLEMENTARY DATA

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- b. Training on animal use
- c. Defining research question and hypothesis
- d. Choosing appropriate methods to address research questions
- e. Optimizing study design
- f. Planning experimental procedures
- g. Handling of animals before the experiment
- h. Handling of animals during the experiment
- i. Handling of animals after the experiment
- j. Deciding humane endpoints
- k. Choosing methods for euthanasia
- l. Reporting and publishing

Response: Tick one box for each statement

5. To what extent do you agree with the following statements?

Scale:

Don't know	Strongly disagree	Disagree	Neither disagree/agree	Agree	Strongly agree
0	1	2	3	4	5

- a. Stressed animals yield less valid results
- b. Complete replacement of the use of animals in research and testing will never be achieved
- c. Complete replacement of the use of animals in education will never be achieved
- d. Researchers are reluctant to change the way they work because of the need for comparability with earlier findings
- e. Environmental enrichment may compromise results
- f. Using computer simulation could one day accurately represent whole animals
- g. Using the 3Rs will be detrimental to the quality of the research results
- h. Using the 3Rs will be detrimental to the quality of the education where animals are used

Response: Tick one box for each statement

II. Implementation of the 3Rs

1. Using the definitions of the 3Rs, which of the 3Rs is most relevant in the **RESEARCH at your university?**

- a. Replacement
- b. Reduction
- c. Refinement
- d. All three are equally relevant
- e. None are especially relevant
- f. I don't know

Response: Tick one option only

2. Using the definitions of the 3Rs, which of the 3Rs is most relevant in the use of animals for **EDUCATION at your university?**

- a. Replacement
- b. Reduction
- c. Refinement
- d. All three are equally relevant
- e. None are especially relevant
- f. I don't know

Response: Tick one option only

3. To what extent do you agree with the following statements?

Scale:

Don't know	Strongly disagree	Disagree	Neither disagree/agree	Agree	Strongly agree
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0 1 2 3 4 5

Implementation of **REPLACEMENT** strategies at your university results in:

- a. Improved animal welfare
- b. Improved scientific quality
- c. Improved educational quality
- d. Further implementation of 3R strategies
- e. Increased bureaucracy
- f. Increased research costs
- g. Slowed down innovation

Response: Tick one box for each statement

4. To what extent do you agree with the following statements?

Scale:

Don't know	Strongly disagree	Disagree	Neither disagree/agree	Agree	Strongly agree
0	1	2	3	4	5

Implementation of **REDUCTION** strategies at your university results in:

- a. Improved animal welfare
- b. Improved scientific quality
- c. Improved educational quality
- d. Further implementation of 3R strategies
- e. Increased bureaucracy
- f. Increased research costs
- g. Slowed down innovation

Response: Tick one box for each statement

5. To what extent do you agree with the following statements?

Scale:

Don't know	Strongly disagree	Disagree	Neither disagree/agree	Agree	Strongly agree
0	1	2	3	4	5

Implementation of **REFINEMENT** strategies at your university results in:

- a. Improved animal welfare
- b. Improved scientific quality
- c. Improved educational quality
- d. Further implementation of 3R strategies
- e. Increased bureaucracy
- f. Increased research costs
- g. Slowed down innovation

Response: Tick one box for each statement

6. To what extent do you agree with the following statements?

Scale:

Don't know	Strongly disagree	Disagree	Neither disagree/agree	Agree	Strongly agree
0	1	2	3	4	5

The following obstacles exist for implementing the 3Rs in research at your university:

- a. Lack of knowledge
- b. Lack of appropriate scientific or technological innovations
- c. Comparability of data

- d. Time/pressure of other duties
- e. Insufficient funding available
- f. Legislation or regulatory requirements

Response: Tick one box for each statement

7. Are you aware of any ongoing or imminent development of 3Rs techniques at your university?

- a. Yes, to replace the use of animals
- b. Yes, to reduce the number of animals
- c. Yes, to refine the use of animals
- d. None of these
- e. I don't know

Response: Tick all that apply

8. What would enable your university to address research objectives **WITHOUT THE USE OF ANIMALS (REPLACEMENT)?**

- a. Nothing, since the researchers at our university need to look at whole animal systems
- b. More relevant cell cultures
- c. Availability of human tissues
- d. Technical advances in tissue engineering
- e. Help in identifying replacement techniques
- f. A system for conducting literature searches for replacement
- g. More predictive computer models
- h. Access to better computing skills
- i. Increased funding to develop alternatives
- j. Legislative or other regulatory changes
- k. Requirements from scientific journals
- l. Something else
- m. I don't know

Response: Tick all that apply

If "Something else", what?

9. Which factors would allow your university to **USE FEWER ANIMALS (REDUCTION) in research?**

- a. More sharing of data or collaboration between research groups
- b. More sharing of data or collaboration between companies (industry)
- c. Greater willingness among researchers to change their methods
- d. Greater availability of funding for 3R research
- e. Changes to legislation or regulatory requirements
- f. Research groups being more willing to accept results obtained using non-animal methods
- g. Greater willingness from regulators to accept data from non-animal approaches
- h. Scientific journals being more willing to accept results obtained using non-animal methods
- i. Breeding programs being conducted by fewer, but larger, specialized establishments
- j. Use of genetically modified animals
- k. Reduced availability of funding for in vivo research
- l. Increased availability of funding for in vitro/in silico research
- m. Something else
- n. I don't know

Response: Tick all that apply

If "Something else", what?

10. Which factors would **IMPROVE THE WELFARE OF THE ANIMALS (REFINEMENT)** used in research at your university?

- a. Greater willingness among researchers to change their methods
- b. Increased funding for the development of refinement methods
- c. Best practices for housing and enrichment for different species, strains, sexes and ages
- d. Standard Operating Procedures for acclimatization and training for every experimental procedure
- e. Technical advances in equipment
- f. Improved marking techniques
- g. Improved analgesia
- h. Increased training of the staff handling the animals
- i. Limits to the number of staff handling the animals
- j. Changes to legislation or regulatory requirements
- k. Requirements from scientific journals
- l. Increased knowledge in ethology (animal behavior) among staff
- m. Increased knowledge of physiology/stress among staff
- n. Something else
- o. I don't know

Response: Tick all that apply

If "Something else", what?

III. Implementation of the EU Directive 2010/63 on the protection of animals used for scientific purposes

1. Does your Animal Welfare Body (AWB) advise the staff on **IN VITRO/IN SILICO METHODS** as **REPLACEMENT** for animal use in research?

- a. In 10 out of 10 projects
- b. In five to nine out of 10 projects
- c. In two to five out of 10 projects
- d. In one out of 10 projects
- e. Never
- f. I don't know

Response: Tick one option

2. Does your AWB advise the staff on **STRATEGIES FOR REDUCTION OF ANIMAL USE** in research (e.g., method developments, coordination with other research groups)?

- a. In 10 out of 10 projects
- b. In five to nine out of 10 projects
- c. In two to five out of 10 projects
- d. In one out of 10 projects
- e. Never
- f. I don't know

Response: Tick one option

3. Does your AWB advise the staff on **METHODS FOR REFINEMENT** (e.g., environmental enrichment, improved handling and experimental techniques)?

- a. In 10 out of 10 projects
- b. In five to nine out of 10 projects
- c. In two to five out of 10 projects
- d. In one out of 10 projects
- e. Never
- f. I don't know

Response: Tick one option

4. To what extent do you agree with the following statements?

Scale:

Don't know	Strongly disagree	Disagree	Neither disagree/agree	Agree	Strongly agree
0	1	2	3	4	5

Your AWB keeps the staff informed of technical and scientific developments within the 3R strategies:

- a. Replacement
- b. Reduction
- c. Refinement

Response: Tick one box for each statement

5. To what extent do you agree with the following statements?

Scale:

Don't know	Strongly disagree	Disagree	Neither disagree/agree	Agree	Strongly agree
0	1	2	3	4	5

Your AWB follows the development and outcome of projects, taking into account the effect on the animals used, and identify and advise on elements that further contribute to the 3R strategies for:

- a. Replacement
- b. Reduction
- c. Refinement

Response: Tick one box for each statement

6. According to EU Directive 2010/63, the AWB should document its advices and keep them open to scrutiny during inspections. Do you think keeping these documents open for other research groups would be beneficial for the implementation of the 3Rs?

- a. Yes.
- b. No.
- c. I don't know.

Response: Tick one option

7. What expertise does your AWB receive input from, with regard to animal welfare?

- a. A designated veterinarian with expertise in laboratory animal medicine
- b. An ethologist (expert in animal behavior) with expertise in the species used
- c. Other expertise in the well-being and treatment of animals
- d. No expertise input is received
- e. I don't know

Response: Tick all that apply

If "Other expertise", what expertise?

8. Does your AWB advise the staff on rehoming animals?

- a. Yes
- b. No
- c. No research animals are rehomed from my university
- d. I don't know

Response: Tick one option

IV. 3R key factors at the university

1. To what extent do you agree with the following statements?

Scale:

Don't know	Strongly disagree	Disagree	Neither disagree/agree	Agree	Strongly agree
0	1	2	3	4	5

The following **KEY FACTORS** are important for successful 3R-work at your university:

- a. Organizational structure
- b. Management
- c. 3R research innovation, validation and implementation
- d. 3R awareness
- e. 3R education and training
- f. Collaboration

Response: Tick one box for each statement

2. To what extent do you agree or disagree with the following statements?

Scale: *Don't know* *Strongly disagree* *Disagree* *Neither disagree/agree* *Agree* *Strongly agree*
 0 1 2 3 4 5

The **AWARENESS** of the 3Rs at your university is increased if:

- a. The management at the university encourages the staff to test 3R ideas
- b. The management at the university sets up annual 3R goals
- c. There is mandatory 3R education (in addition to legislative requirements) for staff
- d. There is a procedure for testing, validating and implementing new methods that results in the 3Rs
- e. There is a 3R policy at the university
- f. The management expects the staff to have high standards with respect to animal welfare and 3R
- g. There is a 3R award at the university
- h. 3R seminars are held on regular basis at the university
- i. There is budget for 3R implementation at the university

Response: Tick one box for each statement

3. To what extent do you agree with the following statements?

Scale: *Don't know* *Strongly disagree* *Disagree* *Neither disagree/agree* *Agree* *Strongly agree*
 0 1 2 3 4 5

The **IMPLEMENTATION** of the 3Rs at your university benefits from:

- a. Increased 3R awareness in management groups
- b. Increased 3R awareness among researchers
- c. Increased 3R awareness among animal technicians
- d. Increased 3R awareness among all staff (equally important for all)
- e. Increased awareness among other individuals/groups in or outside the university
- f. None of the above

Response: Tick one box for each statement

4. To what extent do you agree with the following statements?

Scale:

Don't know	Strongly disagree	Disagree	Neither disagree/agree	Agree	Strongly agree
0	1	2	3	4	5

To increase the implementation of the 3Rs at the university, **RESEARCHERS** would benefit from education and training in the following areas:

- a. Defining research question and hypothesis
- b. Choosing appropriate methods to address research questions
- c. Optimizing study design/statistics
- d. Planning experimental procedures
- e. Reporting and publishing results
- f. Deciding humane endpoints
- g. Choosing methods for euthanasia
- h. In vitro/in silico techniques
- i. Ethology (Animal behavior)
- j. Physiology/stress
- k. Animal housing
- l. Enrichment
- m. Handling of animals before, during and after an experiment
- n. Technical advances in equipment
- o. Improved marking techniques
- p. Improved anesthesia
- q. Improved analgesia
- r. Something else

Response: Tick one box for each statement

If "Something else", what?

5. To what extent do you agree with the following statements?

Scale:

Don't know	Strongly disagree	Disagree	Neither disagree/agree	Agree	Strongly agree
0	1	2	3	4	5

To increase the implementation of the 3Rs, **ANIMAL TECHNICIANS** would benefit from education and training in the following areas:

- a. Animal housing
- b. Enrichment
- c. Handling of animals before, during and after the experiment
- d. Technical advances in equipment
- e. Improved marking techniques
- f. Improved anesthesia
- g. Improved analgesia
- h. Ethology (Animal behavior)
- i. Physiology/stress
- j. Deciding humane endpoints
- k. Choosing methods for euthanasia
- l. Something else

Response: Tick one box for each statement

If "Something else", what?

6. To what extent do you agree or disagree with the following statements?

Scale:

Don't know	Strongly disagree	Disagree	Neither disagree/agree	Agree	Strongly agree
0	1	2	3	4	5

To increase the awareness of the 3Rs at your university, your **AWB** would benefit from collaborating with:

- a. Other AWB's
- b. A National 3R Center (Swedish Board of Agriculture)
- c. Swetox
- d. Ethical committees
- e. Research groups at other universities

- f. External expertise in animal welfare (ethologists, veterinarians, etc.).
- g. Industry
- h. NGO's, e.g., Swedish Fund for Research Without Animal Experiments
- i. Other stakeholders

Response: Tick one box for each statement

If "Other stakeholders", please identify them

Appendix 2: Factor analyses, rotated factor pattern

Factor analyses, rotated factor pattern, including all survey questions with 5-graded response rating from strongly disagree to strongly agree (QI4-5, QII3-5, QIII4-5, QIV4-5). All variables were categorized as replacement-, reduction- and refinement-related responses, and as a more general response including all 3Rs, before performing the factor analyses. In addition, all questions were divided into positive and negative attitude to the 3Rs. The questions were summarized into three factors, i.e., factor 1, 2 and 3, respectively, with the highest loading above the absolute value (i.e., irrespective of its sign) of 0.3 (bolded numbers). Four variables were excluded because they were less than the absolute value of 0.3, or due to less than 0.01 difference between factors (italics).

Variable	Factor 1	Factor 2	Factor 3	Replace	Reduce	Refine	All 3Rs	Positive	Negative
QI 4a	0.67814	0.19210	-0.07037			X		X	
QI 4b	0.49233	0.29508	-0.00901			X		X	
QI 4c	-0.07672	0.09081	0.69505				X	X	
QI 4d	0.07502	0.19563	0.70123				X	X	
QI 4e	0.44227	0.09163	0.53363		X			X	
QI 4f	0.65302	0.21058	0.47758				X	X	
QI 4g	0.52101	0.22981	-0.23830			X		X	
QI 4h	0.45638	0.21926	0.04750			X		X	
QI 4i	0.33109	0.10180	-0.11009			X		X	
QI 4j	0.67811	0.15445	0.03246			X		X	
QI 4k	0.57694	0.45754	0.21348			X		X	
QI 4l	0.09039	-0.06831	0.53254				X	X	
QI 5a	0.35136	0.40084	0.11374			X		X	
QI 5b	-0.43792	0.32356	-0.08351	X					X
QI 5c	<i>-0.21031</i>	<i>0.22673</i>	<i>-0.14488</i>	X					X
QI 5d	0.38984	-0.16640	0.10820				X		X
QI 5e	-0.50503	0.09825	0.15447			X			X
QI 5f	<i>0.15783</i>	<i>0.07480</i>	<i>0.18302</i>	X				X	
QI 5g	-0.54443	0.31685	-0.33444				X		X
QI 5h	-0.61897	0.21513	-0.30292				X		X
QII3a	0.02697	0.20750	0.71042	X				X	
QII 3b	0.13922	0.09935	0.87759	X				X	
QII 3c	0.20421	-0.11310	0.71981	X				X	
QII 3d	0.11641	-0.01002	0.73739	X				X	
QII 3e	-0.62119	-0.28123	-0.19733	X					X
QII 3f	-0.65137	-0.15669	-0.17393	X					X

QII 3g	-0.44546	-0.33022	-0.50623	X					X
QII 4a	0.12949	0.28637	0.62251		X			X	
QII 4b	0.19250	-0.00054	0.74078		X			X	
QII 4c	0.22243	-0.10889	0.68675		X			X	
QII 4d	0.22212	-0.16993	0.87489		X			X	
QII 4e	-0.60688	-0.44641	-0.06775		X				X
QII 4f	-0.55849	-0.33581	0.06860		X				X
QII 4g	-0.60436	-0.45039	-0.30558		X				X
QII 5a	0.96409	0.10821	0.19207			X		X	
QII 5b	0.76077	0.05975	0.24409			X		X	
QII 5c	0.56830	0.05207	0.21579			X		X	
QII 5d	0.57473	0.07287	0.51063			X		X	
QII 5e	-0.51835	-0.17843	-0.06276			X			X
QII 5f	-0.28724	-0.21721	-0.42750			X			X
QII 5g	-0.46621	-0.31415	-0.30093			X			
QIII 4a	-0.18537	0.36939	0.79072	X					
QIII 4b	-0.31839	0.20010	0.69176		X				
QIII 4c	-0.26657	0.18811	0.59042			X			
QIII 5a	-0.72194	0.19958	0.26149	X					
QIII 5b	-0.54837	0.14861	0.46877		X				
QIII 5c	<i>-0.44870</i>	<i>0.12670</i>	0.45898			X			
QIV 4a	0.59090	0.10853	0.63562				X	X	
QIV 4b	0.65523	0.05233	0.45478				X	X	
QIV 4c	0.74158	0.06338	0.34222		X			X	
QIV 4d	0.61422	0.13657	0.45701				X	X	
QIV 4e	0.43104	0.29248	0.63847				X	X	
QIV 4f	0.76399	0.23529	0.30224			X		X	
QIV 4g	<i>0.52335</i>	<i>0.51150</i>	<i>0.50131</i>			X		X	
QIV 4h	0.34148	0.22723	0.52277	X				X	
QIV 4i	0.57331	0.27601	0.38949			X		X	
QIV 4j	0.55609	0.52295	0.32436			X		X	
QIV 4k	0.36187	0.53159	0.21126			X		X	
QIV 4l	0.56008	0.48364	0.26184			X		X	
QIV 4m	0.62200	0.47092	0.30135			X		X	
QIV 4n	0.45190	0.51557	-0.05002			X		X	
QIV 4o	0.44820	0.46865	-0.01199				X	X	
QIV 4p	0.46294	0.54760	0.24027			X		X	
QIV 4r	0.41535	0.63107	0.15060			X		X	
QIV 5a	0.11794	0.87249	0.12504			X		X	
QIV 5b	0.08738	0.87655	0.07468			X		X	
QIV 5c	0.24311	0.89531	0.09450			X		X	

QIV 5d	0.15849	0.84146	0.03659				X	X	
QIV 5e	-0.12532	0.67792	0.20885			X		X	
QIV 5f	0.02571	0.76857	0.15901			X		X	
QIV 5g	-0.00217	0.82481	0.08634			X		X	
QIV 5h	0.11902	0.79180	0.17662			X		X	
QIV 5i	0.00322	0.96068	-0.11503			X		X	
QIV 5j	0.06952	0.93197	0.02705			X		X	
QIV 5k	0.15882	0.77695	0.35752			X		X	