

3R funding of research activities 2014-2019 in Denmark by the Danish 3R centre

Survey of applications

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3R in DK - History

Cell culture work in 80ies

Focus on less use of animals in vaccine testing

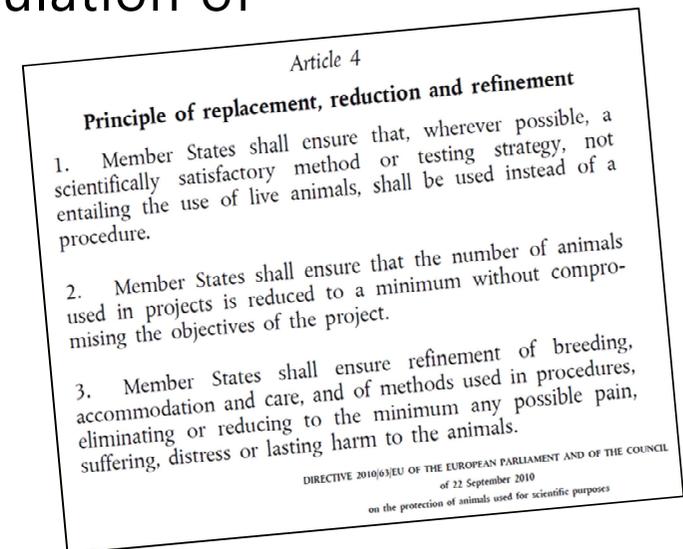
Promotion of the Replacement in EU

- EU research programs – Reprotect, Nanotest mm
- scientific societies DACOPA
- Directive implementation
- 3R centres
- Workshops and knowledge exchange
- National research programs – 3R center

TABLE 1 Timeline of the development and implementation of alternative methods in the Nordic countries

- 1963 The Danish Society for the Protection of Laboratory Animals <http://forsoegsdyrenes-vaern.dk>
- 1964 The Nordic Fund for Research without Animal Experiments <http://www.forskautandjurforsok.se>
- 1971 Juliana von Wendt's Foundation, Finland Juliana von Wendt <http://www.jvws.org/>
- 1981 The first symposium arranged by the Swedish Board for Laboratory Animals (CFN)
- 1983 The Scandinavian Society for Cell Toxicology (SSCT) Björn Ekwall, Erik Walum <http://www.ssct.se>
- 1989-1999 The Multicenter Evaluation of In vitro Cytotoxicity (MEIC) study
- 1996 Nordic research prize for development of alternative methods Research without animal experiments (SE), Juliana von Wendt Fund (FI), Alternativfondet (DK)
- 1997-2001 Oculotox-in vitro Lotta Salminen, Hanna Tähti et al, University of Tampere
- 1999 The EDIT project Björn Ekwall et al
- 1999-2008 The Cell Research Center (CRC) at the Medical Faculty, Univ of Tampere Hanna Tähti Timo Ylikomi
- 2001 Björn Ekwall Memorial Foundation The SSCT <http://www.BEMF.eu>
- 2001 DACOPA Ove Svendsen et al
- 2003 FINCOPA Hanna Tähti, 2003-2008. Tuula Heinonen, 2008-2016, Paula Hirsjärvi, 2016
- 2004 ReProTect Lennart Dencker, Eva Brittebo et al, (SE), Kirsi Vähäkangas et al (FIN), Lisbeth E. Knudsen (DK)
- 2005-2010 The ACUTETOX project Cecilia Clemedsson (SE), scientific coordinator <http://acutetox.eu>
- 2007 Norecopa Adrian Smith <https://norecopa.no>
- 2008 FICAM, the Finnish Center for Alternative Methods at the Univ of Tampere Tuula Heinonen <http://www.ficam.fi>
- 2013 Danish 3R Centre Ministry for Food, Agriculture and Fisheries
- 2014 Swetox, Swedish Toxicology Sciences Research Centre Eleven Swedish Universities <http://swetox.se>
- 2016 Swedish 3R Centre Swedish Board of Agriculture <http://www.jordbruksverket.se/>
- 2017 Finland's 3R Consortium established by TOKES, National Committee for the Protection of Animals used for Scientific and Educational Purposes Finnish Universities, Research Institutions and Industry

- The 3Rs – **R**eduction, **R**eplacement & **R**efinement – will soon celebrate 60 years anniversary
- The 3Rs plays increasingly a role in regulation of animal welfare



- Yet, there is limited knowledge about:
 - Actual implementation
 - Barriers to further implementation

- Research aim: to survey animal researchers knowledge, attitudes and experiences with the 3Rs in Denmark

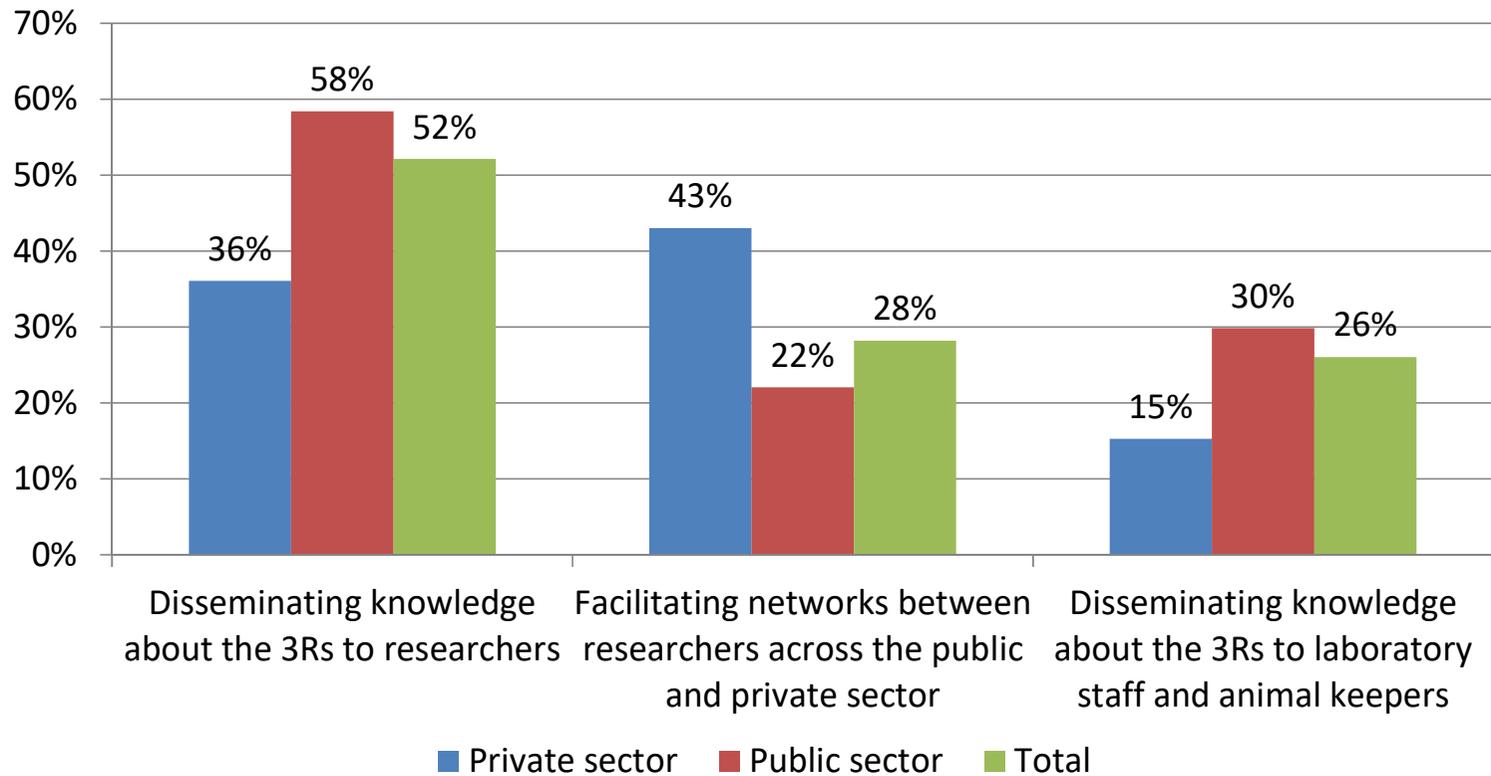
- Project funded by the Danish 3R-Center



The Danish 3R-survey



On which areas should the Danish 3R Center focus its activities?



Another survey - Replacement – options and challenges



- Purpose of the project is to investigate the possibilities and challenges of implementing replacement on private and public research facilities using animals for scientific purposes.
- The project focuses on social and cultural aspects, attitude and level of knowledge impacting the implementation of replacement.
- The aim is to clarify which factors impact the poor implementation of replacement in Danish research communities using animals for scientific purposes.

Meetings



Måned og år	Dato:	Organisation:	Sted:	Deltagere / (kontaktperson):	Formål / Danmarks 3R-Centers mål:	Aftaler:	Opfølgning / initiativ:
2017-10	3.-4. oktober	DCAW / NordCAW		København	Tom Bengtsen, Louise Holm Parby, Rasmus Normann Nielsen	Vidensopdatering, netværk	
2017-8	20.-24. august						
<h2>10th World Congress on Alternatives and Animals in the Life Sciences</h2>							
Erwin L. Roggen, Jan Lund Ottesen, Tom Bengtsen, Louise Holm Parby, Rasmus Normann Nielsen				Vidensopdatering, netværk	Planlagt møde med Svensk 3R-Center i forbindelse med dets åbning i november		Seattle Adrian Smith,
2017-6	30. maj-2. juni	Scandias	København	Adrian Smith, Axel Kørnerup Hansen, Jan Lund Ottesen, Tom Bengtsen, Louise Holm Parby, Kirsten Bayer Andersen		Vidensopdatering, netværk	
2017-5	8.-9. maj	NordCAW	Tartu (Estland)	Tom Bengtsen, Rasmus Normann Nielsen	Indflydelse på udviklingen af regionale dyrevelfærdscentre.	Videndeling på skandinavisk plan	
2017-3	8. marts				NC3Rs	London	Tom Bengtsen Netværk
2017-2 2016-12	22. februar 8. december	AniImpact Webinar ANIMPACT final conference	Online Bruxelles	Jan Lund Ottesen, Peter Bollen, Kirsten Bayer Andersen Tom Bengtsen, Rasmus Normann Nielsen		Vidensopdatering	Webinar februar
2016-12	<h3>EU Kommissionen: Non-Animal Approaches - The Way Forward</h3>				Bruxelles	Lisbeth E. Knudsen, Adrian Smith, Jan Lund Ottesen, Erwin L. Roggen, Tom Bengtsen, Kirsten Bayer Andersen	
2016-12	1. december 2016	NordCAW	Uppsala	Følge udviklingen / evt. nye EU-regler		Indflydelse på udviklingen af regionale dyrevelfærdscentre.	Videndeling på
2016-10 2016-09 2016-09	26. - 28. oktober 2016 Formiddag af centerets arbejde og opbygning med henblik på fremtidigt samarbejde 5.-6. september 2016 1.-2. september 2016	NordCAW Mulighed for afholdelse af NordCAW konference i DK i 2017	Uppsala		Mattilsynet (Norge)	Fødevarestyrelsen	Tom Bengtsen, Rasmus Normann Nielsen, Kirsten Bayer Andersen (Marianne) løbende kontakt og opfølgning Arbejde videre
2016-08	24.-27. august 2016			Adrian Smith, Rasmus Normann Nielsen, Louise Holm Parby	Poster om Danmarks 3R-center, vidensdeling, netværk	Birgitte Kousholt, AU deltog med støtte fra 3R-center for at præsentere succes med dyrevelfærdsorgan. Aftale med EU-kommissionen	Linz
2016-08 Danmark	<h3>"EEMGS3R-tema / ECOPA"</h3>			København	Lisbeth E. Knudsen, Tom Bengtsen	Økonomisk samarbejde / poster om 3R-centerer hele ugen - Vidensdeling, netværk	Kontakt til INERIS / Frankrig vedr. 3R-initiativer i
2016-06	16-17. juni. 2016			Danmarks 3R-Center. Vidensdeling, netværk	<h3>RSPCA International Meeting: Focus on severe suffering</h3>		Bruxelles Peter Bollen, Jan Lund Ottesen Præsentation af
2016-06	13-16. juni. 2016				Bruxelles	Peter Bollen, Erwin L. Roggen, Jan Lund Ottesen, Adrian Smith, Jesper Lassen, Tom Bengtsen, Kirsten Bayer Andersen	Vidensopdatering,
2016-05	31. maj-1. juni 2016					Præsentation af dansk dyrevelfærdsorgan på EUSAAT 2016. Kontakt med kommende belgisk 3R-Center Axel Kørnerup Hansen, Adrian Smith, Tom Bengtsen (Maurice Whelan)	Analyse og beskrivelse af Danmarks 3R-center sendt til Belgien 2. møde ved etablering af samarbejde mellem europæiske 3R-Centre
2016-03	Marts, 2016					EU - kommissionen gennemfører en profilanalyse af alle centre for at dokumentere åbenlyse samarbejdsmuligheder. Tom Bengtsen, Louise Bjørn Brønden (Vicky Robinson)	Inspiration og samarbejde om initiativer som DK3R kan bruge/udvikle/kopiere Aftale om
2015-11	2015-09					inspiration og promovering af NC3R initiativer; CRACK-IT (særligt solutions), ARRIVE, EDA grimace scales - Vurdering af ansøgninger/håndtering af fondsmidler. Bestille materiale til brug ved konference etc. - Tage kontakt til Natalie vedr. EDA, som skal gribes rigtigt an - Ok at kontakte Emily mhp. yderligere info omkr sociale medier etc.	
2015-11	September. 2015					Øget nordisk samarbejde om kommunikation af viden om forsøgsdyr og 3R EUSAAT 3R konference	Endnu en konference planlagt til 2016 Erwin L. Roggen, Lisbeth E. Knudsen, Tom Bengtsen, Rasmus Normann Nielsen
2015-06	19. juni 2015					RIVM	København Christine Nellemann, Lisbeth E. Knudsen, Tom Bengtsen (Anne Keinhuus)
2015-05	20.-21. maj 2015					hollandsk 3R-center	Indsamle og formidle viden om alternativer til dyreforsøg - Samarbejde med lignende centre i udlandet Gensidig dialog og link til hjemmesider Tom Bengtsen (Mats Sjöquist)
2015-04	Forår 2015					Norecopa Oslo	Adrian Smith, Rasmus Normann Nielsen Informationsudveksling via Adrian Smith - Indsamle og formidle viden om alternativer til dyreforsøg - Samarbejde
2015-04	21.-22. april 2015					Swetox	Deltagelse i årsmøde 2014 - Norske 3R-nyheder indgår i nyhedsoversigt. - Danmarks 3R-Center følger op mht. på nyhedsliste Erwin L. Roggen, Adrian Smith, Tom Bengtsen (Ian Colgrove)
2015-04	21.-22. april 2015					Swetox	Diskussion af mulighed for fælles nordisk 3R-samarbejde - Indsamle og formidle viden om alternativer til dyreforsøg - Samarbejde med lignende centre i udlande

Applications for research

The Danish 3R center was established in 2013 and as part of the center activities, research funding is included up to DKK 1.5 million per year with a maximum of DKK 0.5 million per project.

The applications are submitted to the 3R center on a special form and include information on the project's purpose, method, expected significance for the 3R and a clarification of which 3R is sought.

Evaluation

- The research quality of the applications is assessed before an actual research evaluation is made by the board of the 3R center based on the criteria:
- Is the project clearly defined in relation to 3R?
- Is the project's scientific standard suitable for peer-reviewed publication?
- Which of the 3 Rs is covered by the project?

Method for evaluation

Titles and applicants for all applications submitted to the 3R Center during the period 2014-2019 have been available for this inventory, as well as the research assessment carried out by the Research Council in 2014-2017 and then by the 3R Board.

Also the classification of which R application relates to has been available and it is limited by me to two groups Replacement / Reduction and Refinement / Reduction.

Follow-up

The approved projects are regularly followed by the secretariat in the 3R center and all projects have presented at the annual conference organized by the 3R center, several both first with project idea and with results. All approved projects have given feedback on what has been published and this applies to most of the completed projects.

For all projects applied for, I have searched in PubMed for applicants and key words that correspond to the application, thereby identifying publications within the topic. I myself have assessed whether the publication's abstract corresponded to the title of the application.

Number, categorization and success rates for applications submitted to the Danish 3R center in the period 2014-2019

year	2014	2015	2016	2017	2018	2019	ialt
Number of applications	18, 4 supported 22%	21, 3 supported 14%	14, 3 supported 21%	34, 3 supported 9%	18, 3 supported 17%	12, 3 supported 25%	117, 19 supported 16%
Same application resubmitted		2 from 2014	2 from 2015	4: 2 from 2015 og 2 from 2016	7 from 2017	3: 2 from 2017,2018 og 1 from 2018	19, 7 supported 37%
Not research	2	3	3	1	1	1	11, 2 repeats
Replacement/reduction	10	12	6	17	7	3	55
Refinement/reduction	8	9	8	17	11	9	62

Discussion

The survey shows a fluctuation in the number of applications with a boom in 2017 that may be related to marketing?

There seems to be a decrease in applications with replacement as a subject, which may be due to the lack of success rate but real reasons require closer examination.

This is hardly due to lack of interest in the area judged by participation in seminars etc. The success rates of applications for 3R in relation to obtaining support are of the same magnitude as some funds and perhaps even better than applications to the Danish Research Agency.

Discussion

Re-applications have a greater success rate hopefully based on improved applications in relation to posting. The information on the publication has been prepared on the basis of summary material without a close reading of the individual applications and with my estimate on the publication's relation to application based on abstract and title.

Some applications without support have been possible with publications which could be interpreted as an incentive created by the 3R offering. Further data with references to the published works in relevant professional forums may increase the impact of the 3R grant.

Research Funding



2014

- Refinement of animal models of pain: Establishment of strategies to alleviate avoidable pain in rat models for pain and inflammation
- Pathological and immunological consequences of basic experimental animal procedures in mice
- Reducing the group size in studies of dermatitis by standardization of the gut microbiota
- Establishment of an In vitro model for diabetic atherosclerosis

2015

- An in vitro method to predict acute lung toxicity due to pulmonary surfactant inhibition
- A Refined Approach to Producing Polyclonal Antibodies in Chickens – Completely Replacing All Invasive Elements by Combining Immunizations with Routine Aerosol-based Vaccinations
- An alternative to animal experiments: Development of an in vitro human skin model for evaluation of topical antimicrobial compounds

2016

- Development of computer models to predict chemicals interference with thyroid hormones
- Towards better brain cancer treatment with novel in vitro models and fewer animal experiments
- Use of cell culture technology to minimize the needs for animal trials in development and production of fish vaccines

2017

- Use of Primary Isolates From Human Kidney to Study the Molecular Aspects of Blood Pressure Regulation
- Developing FACS based in vitro assays to measure antibody mediated protection against infection with intracellular bacteria
- Implementation of analgesic refinement in rats used as models for arthritis and inflammatory pain

2018

- The mouse pass port
- New advanced blood infection model
- Transport and metabolism of azol antifungal drugs in the human term placenta

2019

- Impact of shelter enrichment of metabolic cages in the studies of protein metabolism
- Genetic modification of mice without the need for extensive breeding
- Investigation and analysis of internal validity in Danish preclinical research

The Swedish 3R-grant in numbers

“Research project grant – Development of methods to replace, reduce and refine animal experiments (3R)”

	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Total
Rejected	23	28	65	45	47	54	56	46	66	41	?	471
Approved	22	8	5	8	7	7	6	6	6	6	?	81
Approval rate (%)	48,9	22,2	7,1	15,1	13,0	11,5	9,7	11,5	8,3	12,5	?	16%
Total	45	36	70	53	54	61	62	52	72	48	65	621

At the Scientific council since 2009. Previously awarded by other authorities since 1989.

The purpose of the project grant is to give researchers the freedom to formulate by themselves the research concept, method and implementation, and to solve a specific research task within a limited period. The focus of this grant is to promote the development of methods for replacing, reducing and/or refining experiments on animals according to the 3R principle.

Bjørn Ekwall Prize

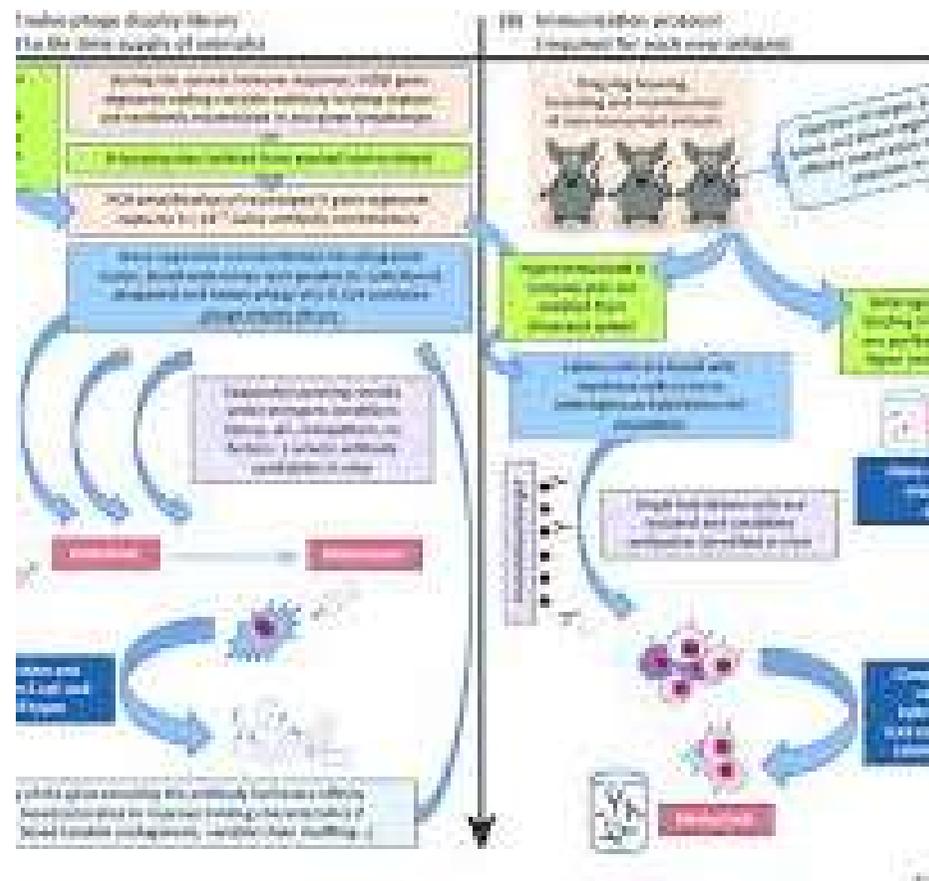
Annual prizes handed out by the
Scandinavian Society of Cell Toxicity

- **Bjørn Ekwall Memorial Award**
to Jan van der Valk, NL-Utrecht
Introduction & Award Ceremony
Stina Oredsson, SE-Lund
Bjørn Ekwall Memorial Foundation
- **Bjørn Ekwall Memorial Award Lecture**
**History of in vitro methods. Lessons
learned?**
Jan van der Valk, NL-Utrecht
Discussion



Nordisk Forskningspris

- 2019 Prisen blev tildelt Dr. Alison Gray, Afability, Nottingham University
- Øge forståelsen i forskerkredse for at skiftet fra at fremstille antistoffer med brug af dyr til at fremstille dem med dyrefri metoder



Non Technical summaries

- Denmark 2017:

- "...Unfortunately it requires a complete immune system to be able to form antibody-producing plasma b-cells. There are alternative methods of producing antibody fragments (not intact antibodies), but these are not yet at a point where they can substitute hybridoma technology for production of antibodies with high affinity and specificity...."

"....Formation of polyclonal antibodies can only happen in living animals, as it requires a functional immune system. Today there are in vitro methods for generation of antibodies, but these do not measure up to polyclonal antibodies from animals, with regard to either specificity or affinity. In the animals a maturation of the antibodies takes places, which cannot be achieved without a complete immune system...."

".... Through immunisation the animal develops a wide repertoire of antibodies against the given antigen and the individual animals each make a unique and different repertoire of antibodies against the same antigen. This cannot be achieved through use of artificial antibody libraries, which have a limited and fixed repertoire. There is therefore no corresponding ex vivo technology/replacement which gives antibodies of the same quality, specificity and affinity...."

"...To form new antibodies, it is necessary to provoke an immune response against a given antigen in a live animal. ... plasma cells (derived from B-cells) form antibodies to the injected antigen. These hybridoma are then used for large scale antibody production in vitro. As we wish to form new antibodies, it is not possible to carry out this experiment without live animals...."

-

In EU applications justification is requested

Researchers should provide details of the species (and strains where appropriate) of animals to be used and explain why they have been chosen. They should explain why the anticipated benefits justify the use of animals and why methods avoiding the use of living animals cannot be used. They should also give details and justify the numbers of animals proposed with reference to statistical advice if applicable.

They have to indicate what steps have been taken to comply with the principles of the 3 Rs: reduction, refinement and replacement. In particular, they should describe these procedures adopted to ensure that the amount of suffering to the animals is minimised and that their welfare is protected as far as possible (e.g. improvements in technique, application of human end-points, environmental enrichment).

- To Check for **alternatives** (cf. the following websites):
 - <http://www.nc3rs.org.uk/category.asp?catID=3>
 - http://www.vet.uu.nl/nca/links/databases_of_3r_models
 - <http://ecvam.jrc.it/>