The role of review and regulatory approvals processes for animal research in supporting implementation of the 3Rs

A report by Dr Frances Rawle,
commissioned by the NC3Rs

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About Dr Frances Rawle

Frances has extensive experience of managing scientific peer review and of research policy, research ethics and governance throughout a long career at the Medical Research Council (MRC).

For many years Frances was responsible for policy on animal research and the MRC's funding of the NC3Rs, and from 2018 until 2021 she represented the MRC on the NC3Rs Board. Frances has a particular interest in research integrity and reproducibility. Frances now works as an independent consultant and was on the Steering Group for the ASRU Change programme in 2021/22.

Foreword

In the UK oversight of the use of animals for research purposes in the academic sector is undertaken by various bodies and at various stages.

This includes review by public and charitable funding bodies, the national regulator and locally by ethics committees. The 3Rs are on the face of it at least an important consideration in the review by all of these organisations. This means that inevitably there is the potential for overlap and duplication of efforts. That said, there is often a long lag between the development of 3Rs approaches and their use in routine practice, even for simple advances that benefit animal welfare.

The current oversight mechanisms should support the NC3Rs mission, but it is not clear that this is happening to the extent it should be despite the academic community's long-standing commitment to the 3Rs. To try to address this and to identify gaps and overlaps the NC3Rs commissioned Dr Frances Rawle to undertake a detailed and independent review, including engagement with key stakeholder groups.

Dr Vicky Robinson,
NC3Rs Chief Executive

Executive summary

Background

The 3Rs principles – replacement, reduction and refinement – are the widely accepted ethical framework for the use of animals in research, and compliance with these principles is a legal requirement in the UK under the Animals (Scientific Procedures) Act 1986 (ASPA).

Obtaining a project licence (PPL) under ASPA requires review by an Animal Welfare and Ethical Review Body (AWERB) at a Home Office licenced establishment and by the Animals in Science Regulation Unit (ASRU) Inspectors, to establish (amongst other things) whether the proposed research complies with the 3Rs principles. In addition, most academic research involving animals is subject to peer review by public sector or charitable funders. Most funders are committed to promoting the 3Rs and their peer review covers relevant areas, but the extent to which implementation of the 3Rs is explicitly considered varies. The focus of funder peer review is mainly on the quality of the proposed research and the likelihood of achieving significant scientific advances.

Aims of the project

The project had three main objectives focusing on academic-led research involving animals in the UK:

1. To map in detail what the various regulatory and review processes and bodies currently do to ensure compliance with 3Rs principles and to promote adoption of 3Rs advances.

2. To identify any current variations in review processes, any gaps (or overlaps) in coverage and any lessons to be learned from examples of particularly effective practice.

3. To explore opportunities for adjusting current processes and responsibilities so as to cover any gaps, remove unnecessary duplication and more effectively promote adoption of 3Rs advances.

Project approach

Information was obtained from interviews (~40) with stakeholders[1] involved in regulatory and review processes, including chairs and members of AWERBs, Establishment Licence Holders (ELHs), Named Animal Care and Welfare Officers (NACWOs), Named Veterinary Surgeons (NVSs), Named Information Officers (NIOs), former and current Inspectors, Animals in Science Committee members, representatives of charitable and public sector funders, and senior scientists with experience as reviewers on funding panels and as holders of PPLs and personal licences. ASRU provided written responses to questions and three AWERB meetings were observed.

Summary of findings

Replacement

Replacement does not seem to be covered well by any of the review processes. AWERBs and ASRU Inspectors rarely suggest use of replacements. They do not (and could not) have sufficiently detailed knowledge of the full breadth of the scientific areas they need to cover to know for every application whether appropriate and practicable replacement technologies are available. AWERBs may assume that by the time a licence application is submitted to them for review the researcher and the funder have considered the options for replacement and concluded that animal use is necessary.

Funders' peer review involves more specialist scientific expertise, but their review tends not to focus explicitly on whether suitable replacements might be available but rather on whether the applicants' chosen models will allow them to answer the scientific questions posed. Where the research is disease focused, the key question for peer reviewers is the relevance of the animal model to the human disease and how likely the results are to translate rapidly into clinical benefits. All funders require applicants to justify the need to use animals and their choice of species, but the extent to which this is challenged by reviewers varies between funders.

Reduction

Both AWERBs and funders report paying closer attention to experimental design and statistics in their reviews over recent years, although AWERBs report a shortage of people with the necessary expertise to review this area. The funders' aim is to ensure the research they are funding is robust and reproducible, which should lead to reductions in overall animal use, although paradoxically the review of experimental design often indicates that more animals are required for each experiment to achieve sufficient statistical power.

It is not possible to review the design of every experiment covered by a grant or a PPL application covering three to five years at the outset, and both PPL and grant reviews focus on typical or early experiments. ASRU reviews the basic principles of experimental design but does not undertake a detailed assessment of the proposed statistical methods. As part of the new audit process, ASRU Inspectors will also evaluate the systems in place at licensed establishments to promote the use of appropriate experimental designs and statistical methods and the availability of local expertise. The NC3Rs Experimental Design Assistant was designed to address the shortage of expert advice in this area but is not yet widely used in grant or PPL submissions. Some establishments review experimental design as part of individual study plans, but the shortage of available expertise is likely to prevent this being done more widely.

[1]See a full list of contributors in Annex 1.

Efficient colony management and breeding are proven means of reducing animal use, and it is important for reproducibility to avoid genetic drift. These aspects are rarely covered as part of project review by either AWERBs or funders, but oversight of colony management and breeding strategies for genetically altered animals at a facility-wide level should be included in the AWERBs other functions.

ASRU asks for information in PPL applications to assess efficiency of breeding, and use of best practice in breeding is reviewed as part of the recently introduced audit process. AWERBs may also oversee a local system to make best use of tissues from culled animals in teaching and research as part of their wider role in promoting the 3Rs.

Neither AWERBs nor funders reported much discussion in their reviews of the potential to use methodological advances such as in-cage monitoring, microsampling, or use of imaging techniques to enable more information to be obtained from fewer animals.

Refinement

Refinement is the area in which AWERBs are most confident to challenge when they review PPL applications and feel that their input adds most value. NACWOs and NVSs usually provide input on the refinement of protocols both in the preparation of licence applications and as a project progresses.

Refinement in housing and husbandry, such as environmental enrichment, is usually not covered as part of the PPL review, but is overseen by the AWERB, the NACWO and the NVS on a facility-wide basis.

Funders' grant reviews occasionally look at refinement (for example with protocols involving severe levels of animal suffering or for specially protected species, when the NC3Rs normally provides an additional welfare review). A small proportion of PPL applications are referred to the Animals in Science Committee, and their reviews may cover refinement. Funders rarely consider housing and husbandry, except in cases where it is critical to the experiment (for example, in studies of the gut microbiome). They felt they could and should rely on AWERBs and ASRU to ensure appropriate refinements were in place.

Barriers to uptake of 3Rs advances

Reasons mentioned by stakeholders for slow uptake of 3Rs advances included:

1. The time and cost involved in setting up new techniques in a laboratory, and lack of access to expert help. The laboratories which have developed new techniques do not have the time and resources to help everyone who wants to try them. Researchers may be concerned that their lack of expertise might make a grant application involving a new method uncompetitive, and that delays in producing data and publications while they get a new model established will negatively affect their career.

2. Lack of published data on how results using replacement technologies compare to established animal models and concerns about acceptance for publication or (in work to develop treatments) by the regulator, for example if there is an accepted "gold standard" animal model in the field. Many researchers think that they must use an animal model because a paper using a new *in vitro* model on its own will not be accepted by the scientific journals.

3. Concerns that introducing refinements to experimental protocols will result in a lack of compatibility with earlier data.

4. Poor access to information on 3Rs advances. Many stakeholders highlighted the need for better availability of credible sources of information on advances in all three 'Rs' for researchers, committee members, reviewers and named persons, including information on evaluation and validation of new methods and on approaches that had been tried and not proved useful. The need to better define and resource the role of the NIO was highlighted, in order to help researchers and AWERBs to access information.

Recommendations

1. Funders should make best use of their access to highly specialist scientific peer reviewers to ensure that possibilities for use of replacements or new approaches to obtain more information from fewer animals are identified and implemented where appropriate. This could be facilitated by using more specific questions for reviewers on whether there are available alternatives and/or reduction strategies.

2. Funders could introduce more targeted questions for applicants to elicit information on replacement and reduction, and guidance for applicants on expectations, with the assumption that in most cases[2] optimising refinement will be ensured by ASRU and AWERB oversight.

3. Funders should be prepared to provide additional funding to allow grant holders to explore and validate the use of new alternatives alongside their established models, and to facilitate dissemination of new methods[3] by supporting laboratories which have developed them to provide access to the technology and train others to use it.

4. It should be made clear in a PPL application what parts of the work have already been funded (including date of award and duration) and by whom, so that AWERBs and ASRU are clear what has been externally peer-reviewed and what has not. Funders should be willing to share information on whether their expert review has explicitly considered whether replacements are available.

5. Establishments should ensure that their processes allow the use of animals to be challenged early in the research planning process. AWERBs should ask questions about whether/how an applicant has searched for information on possible replacements or reduction strategies. They should expect a clear explanation of what replacements have been considered and why they are not suitable, and whether approaches to get more information from a group of animals have been considered. This could be facilitated by guidance to AWERBs on questions to ask and what should reasonably be expected of applicants.

6. Best practice for induction for AWERB members should include training in the 3Rs and the principles of experimental design. The introduction of audit processes in ASRU's new ways of working provides an opportunity to clarify expectations for training of AWERB members and to confirm via audit that these are being followed. In the longer term the requirement for CPD for all AWERB members should be considered by the sector, in line with the Research Ethics Committees which cover projects involving human participants.

[2] Exceptions might be for specially protected species and/or severe protocols or work to be done in another country.
[3] There are opportunities for partnership funding with the NC3Rs.

7. AWERBs should be clear on the expectations for their role in promoting the 3Rs on a facility-wide basis outside the process of PPL review, including the importance of spending enough time and attention on this part of their role and what constitutes good practice. Areas to cover include refinement of housing and husbandry, efficient colony management and breeding, good experimental design, tissue sharing and sharing of 3Rs advances.

8. The expectations of the NIO role should be set out clearly at each establishment in line with ASPA and LASA/IAT guidance[4]. establishments must ensure that NIOs have the expertise, time and appropriate resources and training to effectively support researchers, AWERB members and animal facility staff in accessing information on 3Rs advances. They should be well trained in approaches to search for information and have time to support researchers to fulfil their responsibility to look for alternative approaches. ASRU should cover the effectiveness of the NIO role in their audits.

9. To facilitate access to information about 3Rs advances, the NC3Rs, scientific or learned societies and/or funders should convene expert groups to review information on 3Rs advances available in particular scientific areas or for commonly used animal models of disease, to produce authoritative, up-to-date and easily accessible information for researchers, peer reviewers and AWERBs. Funders should ensure that this information is taken into account in their funding decisions.

10. All AWERBs and funder review panels should have access to expertise in statistics and experimental design. Inventive solutions may be necessary to make best use of available expertise for reviewing given the shortage. The NC3Rs Experimental Design Assistant (EDA) should be more widely used in applications; this may require further development to make it more accessible. With the current focus on improving reproducibility across the life sciences, funders and universities should explore means to support development of more experts in statistics and experimental design, both to help and train researchers on the ground and to participate in expert review.

11. ASRU and AWERBs should ensure that information on 3Rs advances obtained from retrospective reviews and retrospective assessments of PPLs is available to the research community, whether via publication or some other means[5].

12. To reduce unnecessary bureaucracy funders can rely on AWERBs and ASRU for checking implementation of refinement and on ASRU to monitor compliance with ASPA (for example, it is not necessary to include this in funder assurance checks or to ask for formal confirmation of licences before grant funds are released). However, it remains important for funders to check that AWERBs have reviewed any animal research that falls outside of the ASPA, such as work taking place overseas.

[4] Guiding Principles for Named Persons | LASA.
[5] For example, a repository that is easily accessible and searchable.

Detailed Report

Background and context for the project

The 3Rs principles – replacement, reduction and refinement – are the widely accepted ethical framework for the use of animals in research, and compliance with these principles is a legal requirement in the UK under the Animals (Scientific Procedures) Act 1986 (ASPA).

Obtaining a project licence (PPL) under ASPA requires review by an Animal Welfare and Ethical Review Body (AWERB) at the establishment where the research will take place and by the Animals in Science Regulation Unit (ASRU) Inspectors, to establish (among other things) whether the research complies with the 3Rs principles.

Named Animal Care and Welfare Officers (NACWOs) and Named Veterinary Surgeons (NVSs) have local responsibility for animal welfare and a key role in promoting the 3Rs (especially refinement) and Named Information Officers (NIOs) have a responsibility to help researchers and animal facility staff access information about the 3Rs that might be relevant to their work.

Assessment of 3Rs compliance used to be covered in ASRU inspections. ASRU is currently undergoing a major change programme, with the aim of improving its efficiency and effectiveness as a regulator; Inspectors are no longer assigned to specific establishments and inspections have been replaced by a programme of facility, systems and thematic audits. This represents both an opportunity and a risk – the opportunity for ASRU to set out clear expectations of establishments for what they should be doing to promote the 3Rs which they will audit against, with the risk that the focus is on box-ticking rather than ensuring a culture of genuine commitment to advancing the 3Rs.

Most academic research involving animals is externally funded from public sector or charitable bodies, although the research covered by a PPL and by a research grant are rarely the same – one PPL often covers work funded from several different grants and the time periods covered by licences and grants are normally different. Funding is usually subject to peer review processes focused on research quality and the likelihood of achieving significant scientific advances, although research funded from internally managed resources may not be subject to such detailed scrutiny.

Although funders have no legal responsibility under ASPA to promote the 3Rs, all funders interviewed for this study have a commitment to do so. However, the extent to which the 3Rs are explicitly considered in their peer review processes varies. There are concerns that the ASRU and AWERB review processes may involve assumptions that funders' scientific peer review addresses aspects of the 3Rs such as experimental design or the potential for replacement.

Conversely, funders may assume that implementation of the 3Rs is ensured by the AWERB and regulatory review processes. There is also concern that the predominance of well-established senior researchers in peer review may lead to a bias towards use of well-established and familiar animal methods. This project aimed to explore whether these concerns were justified.

With the publication of the *Independent Review of Research Bureaucracy*[6] funders will be reviewing their application processes to reduce unnecessary bureaucracy; it is important that any forthcoming changes to either funder reviews or ASRU regulatory processes do not result in gaps in coverage of the 3Rs.

Methodology

Interviews (~ 40) with people[7] involved in AWERB, funder and ASRU review processes were conducted via video conferencing and lasted about an hour. They followed a topic guide that included questions about the details of the review processes and specific questions about how each of the 3Rs was dealt with.

Interviewees were also asked about barriers that they had encountered (actual or perceived) to implementation of 3Rs advances. Questions were modified to suit the experience an interviewee had of the various review processes. Any interesting observations relevant to the project were followed up by further *ad hoc* questions before returning to the prepared question set. Interviewees were selected to cover a variety of perspectives and experiences of the review of grant and PPL applications. Funder interviews included seven charities of varied sizes (annual research budgets ranging from £2.5M to >£1bn) and the two UK Research Councils that fund the most animal research, the Medical Research Council (MRC) and Biotechnology and Biological Sciences Research Council (BBSRC). They involved staff with experience of peer review processes, panel discussions and funder policies on animal research. Interviews with senior researchers provided an alternative perspective on expert peer review processes.

All the AWERB members (lay and expert) and chairs interviewed had experience in academic establishments, several with more than one AWERB, and a few also had experience of AWERBs in private sector establishments. Many people interviewed had experience of several aspects of these review processes – for example academics who undertook peer review for funders and had their own PPLs reviewed by AWERBs, or people with experience as ASRU Inspectors, researchers and NVSs.

The head of ASRU and the head of the new Animals in Science Policy and Coordination Unit contributed at the start and towards the end of the project, and ASRU provided responses to questions in writing. Three AWERB meetings at which PPLs applications and amendments were reviewed and reports relevant to the implementation of the 3Rs were considered were also observed.

[6] Independent Review of Research Bureaucracy: final report.
[7] Annex 1. In a few cases more than one person participated in an interview, so the number of people is slightly higher than the number. of interviews. One interviewee chose to remain anonymous.

Findings – who is doing what?

Funder peer review

Context

All funders point researchers to the document "*Responsibility in the use of animals in bioscience research*"[8] and emphasise in their guidance the requirement for researchers to implement the 3Rs. All the charities interviewed are members of the Association of Medical Research Charities (AMRC) and subscribe to their position statement[9] on animal research, which includes the 3Rs.

All funders include specific questions for grant proposals involving the use of animals which cover information relevant to the 3Rs, and many mentioned trying to standardise these questions across funders, facilitated by the NC3Rs. Most funders said they have guidance and/or specific questions for reviewers and panel members related to aspects of the 3Rs.

All funders use a combination of written reviews and panel meetings for their expert peer review and decision-making processes, but exact details vary between funders and between different schemes (for example, written reviews may be sought before or after a short-listing step, sometimes it is panel members that provide written reviews, fellowship awards often involve interviews while project grants usually do not). Most funders mentioned having increased their focus on experimental design and statistics in recent years, requiring more information on this from applicants and more scrutiny by reviewers and/or panels. Several funders reported they had experienced increased demand for funding for animal research overseas recently, either for academic collaborations or for preclinical testing by contract research organisations based overseas. They require researchers and their local AWERB to satisfy themselves that welfare standards are equivalent to the UK, and most mentioned use of the NC3Rs checklist[10] for that purpose. A few described requiring specific justification for doing work overseas rather than in the UK, to ensure researchers are not just looking for a way of doing work more cheaply or to do experiments that would not be permitted under UK regulations.

General points

The extent to which the 3Rs are covered in peer review is dependent on the quality of written reviews – although there are questions relevant to the 3Rs not all reviewers answer them. Time constraints are an issue in Panel/Board meetings; around 10 to 15 minutes per application is generally allowed for discussion, which does not allow time to cover all 3Rs issues in detail. Often the focus is more on the scientific ideas and how they will advance the field.

Replacement

Most funders reported that peer review focuses on whether the applicants are using an appropriate model to address the question they want to answer where the use of animal is proposed, rather than on the availability and suitability of replacement technologies *per se*. All funders rely on reviewers and panel members to identify potential replacements based on their knowledge of the field. None reported any systematic searching for possible replacements. Disease-focused charities said that the key issue for their panels is the relevance of the animal model to the human disease and how likely the results are to translate rapidly into benefits for patients.

[8] Responsibility in the use of animals in bioscience research | NC3Rs.
[9] Position statement on the use of animals in research | AMRC.
[10] New checklists to support the assessment of welfare standards in overseas research | NC3Rs.

All funders ask applicants to justify the need to use animals and their choice of species. Answers varied as to how frequently the need to use animals is challenged in their review processes – from "often" or "rigorously" to "rarely". Some funders report challenge from panels on whether all the animal experiments are necessary. Some disease-focused charities reported regular discussions of whether the research should be done in humans or using human tissue or induced pluripotent stem cell-based models rather than animals.

Some funders highlighted that panels will look carefully at whether animals are needed for each part of a programme and may decide animal use is not appropriate for certain parts or may ask for the *in vitro* or *in silico* part of the project to be done first before agreeing to fund subsequent *in vivo* work. Most funders reported increasing numbers of applications wanting to use organoids and *in silico* modelling.

Reduction

Most funders reported an increased level of scrutiny of experimental design and statistics in their peer review. Some use statistics experts on panels or as reviewers to scrutinise this area specifically, others rely on panel members with experience of animal models to do this. Several funders point to the NC3Rs Experimental Design Assistant (EDA) in their guidance for applicants, but the use of its outputs in funding applications is not yet common.

Some funders commented that it was common for scrutiny of experimental design to raise concerns about under-powered studies and identify the need for more animals per experiment to achieve robust results. Funders recognise that for three or five-year programmes of work it is impossible to scrutinise the design of every experiment; they are looking for evidence from a design for an early or typical experiment that applicants know what they are doing, and how they will use information from early experiments to inform the design of later ones.

Most funders do not scrutinise breeding strategies or colony management in their peer review (some funders said they did so for applications involving the development of new transgenic lines). In general, funders felt that this is best done locally, overseen by the AWERB. Some funders said that applicants mention sharing of tissues from otherwise unused animals in transgenic breeding programmes as a reduction strategy, or specifically said that they have funded grants using such material. There was very little mention of other methodological advances that could reduce animal use, such as longitudinal imaging or in-cage monitoring.

Refinement

Refinement is rarely covered in funder peer review. The exception is for specially protected species (and in one case pigs) where funders use the NC3Rs review service to obtain a welfare and 3Rs review. Some funders also use this service for overseas work, or where there are specific concerns identified (such as very large numbers of animals or protocols likely to cause severe suffering). One scientist panel member said that these NC3Rs detailed reviews were very helpful in ensuring 3Rs advances were implemented and should be done for all applications involving animals. However, staff from funders said that the workload involved in doing this would be impracticable due to the high volume of applications involving animals. All funders consider that responsibility for ensuring best practice in housing and husbandry and environmental enrichment should sit with AWERBs and ASRU and should not form part of funder review. The only instances where housing and husbandry might be discussed during grant review would be where this is critical to the experimental design, for example in studies of the gut microbiome.

Project/protocol specific refinement is rarely, if ever, discussed by funder panels. The exceptions mentioned were for work taking place overseas (and thus not covered by UK regulation), disease models rated "severe" and areas of research where there are newer animal models being developed to replace ones with more severe harms.

Several funders specifically said they relied on AWERB and ASRU review and local processes to ensure refinement of experimental protocols was optimised. Most thought that this is where responsibility for promoting refinement should lie.

AWERB review and associated local processes

Context and general points

Flexibility in the implementation of the AWERB functions and the fact that responsibilities are not set out in detail in law means there is a lot of variation in practice, both in how PPLs are reviewed and in how other tasks of the AWERB are carried out.[11] Apart from the smallest establishments, a commonly expressed concern was that the high workload of PPL review meant that AWERBs did not have sufficient time for other functions related to promoting the 3Rs. One establishment reported trialling separate meetings for PPL and other AWERB business, to ensure the latter got adequate time and attention.

Several people commented that the length of PPL applications meant a very high reading workload for committee members, and that there was little recognition or reward for being an AWERB member in academic establishments. The high workload is also a factor in the difficulty AWERBs experience in recruiting lay members who are independent of the establishment (many lay members are university staff from departments not involved in animal research). Establishments covered in interviews for this project ranged from ~15 to >150 PPLs in place at any one time, such that the annual workload of licence review varied widely. The extent to which AWERB work was delegated to subcommittees varied but this was not always related to the size of the establishment. There were some suggestions that scientists who are PPL holders sitting on the AWERB may be reluctant to challenge their colleagues robustly because they know their own licences will be coming round for review in due course.

Several people commented that the NIO role is not well defined and often not well resourced. Many NIOs do the job part-time alongside other busy roles and may lack sufficient training in how to search effectively for information to be able to support licence applicants in finding information on potential replacements, reduction strategies or refinements that may be appropriate in their research.

AWERB review processes differ depending on a range of factors but a number of themes emerged during the interviews.

[11] Guiding principles on good practice for AWERBs | RSPCA and LASA.

The common elements of all AWERB PPL reviews are pre-review input, member comments and a process for applicants to respond to the comments. However, there is a lot of variation in how these elements are implemented. All establishments offer some form of input from the NVS, NACWO and director of the animal facility prior to submission of a PPL application to the AWERB, with processes ranging from provision of written comments on a draft of the application, through informal meetings, to formal meetings with a subcommittee of the AWERB including an NVS and a NACWO.

In some establishments these preparatory meetings are a requirement for all licence applications including renewals, while in others the focus is more on new licences. In some places, the pre-submission input is optional while in others there is a formal process for all applications. The timescale mentioned was usually to start preparation at least six months before submission to ASRU. One person said that the AWERB chair meets prospective new licence applicants before starting the application process, and one mentioned that new applicants do a presentation on their work to the AWERB before preparing their application, to enable the AWERB to identify any ethical concerns. A few people mentioned that pre-application meetings with the assigned Inspector had been a useful part of the preparation process prior to the ASRU reforms.

A few establishments reported having a formal AWERB subcommittee to review PPL applications, or putting together a subcommittee for each application, with expedited discussions in the AWERB meetings focused on minutes or reports from that subcommittee. Some AWERBs use an online discussion forum or email to comment on applications before the AWERB meeting, in which case applicants can review comments in preparation to respond at the meeting or even respond online. AWERBs vary in whether the applicant is required to attend the AWERB or the PPL subcommittee meeting in person and, if they do attend, whether they give a formal presentation or simply answer questions. Sometimes only new applicants are required to attend in person.

In some establishments there is a requirement to submit a study plan for each new study under a licence. This process is generally managed by the animal facility and not by the AWERB. The focus varies; sometimes there is a detailed scrutiny of the experimental design, in many cases the NVS reviews the protocols to ensure refinements are appropriate, including humane endpoints, and facility staff usually check for compliance with licences and/or whether the facility has the resources and appropriately trained staff available to support the planned experiments.

One person mentioned that the information required in this study plan is based on the ARRIVE guidelines, to make sure all requirements for publication have been thought about before an experiment starts. Establishments that require individual study plans find them valuable, and one person said it means they worry less about detailed scrutiny of experimental design at the licence application stage. However, one person commented that their establishment had decided not to introduce them because they are a lot of work to prepare and review and there was no evidence that they lead to fewer instances of non-compliance.

Replacement

AWERBs rarely challenge animal use *per se*. Several people reported that by the time a PPL application comes to AWERB the need to use animals is a given, though they might challenge the need for animal use for certain experiments within the licence. Some mentioned that it is particularly difficult to challenge animal use when it is an ongoing programme being renewed. Some people commented that the time at which animal use should be challenged is in the preliminary discussion for a new licence with the facility manager, the NACWO and the NVS, although they are unlikely to have sufficiently detailed knowledge of replacement methods to do this.

Some AWERB members commented that, due to the expense and difficulty of doing animal experiments, they would expect the applicants themselves to have carefully looked for any possible replacements. Similarly, there was an expectation that funder peer review would have identified opportunities to replace animal use. Many people highlighted that detailed specialist knowledge of a scientific field is required to know whether suitable replacement technologies are available, validated and practicable to implement, and that AWERB members rarely have this level of knowledge, certainly not in all areas of research undertaken in an establishment. Similarly, NVSs said that they do not have sufficient expertise in replacement as their expertise is primarily with animal methods. Therefore, when AWERBs review a PPL application they rarely suggest use of replacements and find it difficult to challenge applicants if they say possible replacements are not suitable.

Many people also commented on the difficulty of accessing information about possible replacements, and the role of NC3Rs (via the Regional Programme Manager where there is one) in this and whether NIOs have sufficient skills to help applicants search for information.

Reduction

A shortage of biostatistical and experimental design expertise available to support licence applicants and to review PPL applications was mentioned by many people. Not all AWERBs include members with this expertise. AWERBs that do have access to statistics and experimental design expertise find it very useful for their PPL reviews. Some people mentioned the need for additional funding for statistical support for researchers.

AWERB members noted that scrutiny of experimental design is easier now that there is a section for relevant information on the licence form. It is not however possible to include the detailed design of experiments to be done five years ahead, so the review must look for evidence that the design of early or typical experiments is robust and information on how the data from earlier experiments will inform the design of later ones.

Some licence applicants use the EDA in preparing their applications, but others find it too time consuming, difficult to use or not appropriate for their experiments. AWERB members pointed out that it is insufficient for applicants to say they have used the EDA, they need to provide an example of an experiment they have designed using it. People commented that individual study plans (where used) are the appropriate level for effective scrutiny of experimental design, but this is only possible where sufficient expert resource is available.

AWERBs rarely review colony management and breeding strategies as part of the PPL review, but sometimes have an oversight mechanism for this as part of their wider role in promoting the 3Rs. Opinion was divided as to whether more attention to this would result in significant reductions in

numbers of animals – many people thought high animal maintenance costs were a powerful driver towards efficient breeding and colony management and overbreeding was no longer a significant problem, while others thought there were still gains to be made. One person commented that too much pressure to reduce numbers can be unhelpful because it encourages researchers to cut corners with quality controls in breeding to prevent genetic drift (for example not back-crossing lines) leading to problems with reproducibility

It was pointed out that some wastage of animals arises when lines needed to be kept "on-the shelf" during the process of writing and review of scientific papers in case additional experiments are requested by reviewers.

Methodological advances such as in-cage monitoring, microsampling, and use of imaging techniques were mentioned as ways to enable more information to be obtained from fewer animals, and one person said AWERBs should look for possibilities to use these during PPL review. However, these technologies are not always available in establishments, and the equipment can be expensive.

Refinement

In most places a lot of work is done on protocol specific refinement such as anaesthesia, analgesia and humane endpoints by NVSs and NACWOs before an application gets to the AWERB. Refinement is the area on which AWERBs are most confident to challenge when they review licence applications and feel that their input adds value. Refinement should be an ongoing process and AWERBs expect to see evidence of ongoing refinement at mid-term and retrospective reviews. However, at least one researcher stated that when a laboratory has been using a model for some time and done a lot of work on refinement already, finding further refinements is difficult. Housing, husbandry and enrichment are mainly dealt with outside the PPL review process by facility staff and NACWOs, and in many establishments this is overseen by a 3Rs committee (in some cases this is a formal subcommittee of the AWERB). These committees may be involved in setting-up studies to evaluate welfare refinements and developing establishment-wide policies and standards for approval by the AWERB. Where there are establishment standards, the AWERB expects any proposed deviation from these to accommodate experimental requirements to be justified in a PPL application or amendment.

Many people commented on the need for better sharing of knowledge and best practice in relation to both refinements specific to particular animal models and to housing, husbandry and other welfare improvements. Of note was a comment on the lack of shared information on approaches to refinement which had been tried and shown not to be effective.

ASRU review

Context and general points

ASRU is bringing in fundamental changes to its operating model underpinned by strategic shifts that are aligned with the Regulators' Code. The new ways of working include a greater focus on the assessment of the suitability of all licence holders, including standards for licence holder training, and an increased focus on legal requirements in the assessment of PPLs. Inspectors are no longer assigned to establishments and PPL applications and amendments are reviewed on a first come, first served basis through a team of dedicated Inspectors. As part of regulatory reform ASRU soon expects to develop and publish new quality standards for licence review.

Licensing functions and compliance assurance are now separated, with the latter including provision of facility, systems and thematic audits, enforcement activities investigating potential cases of non-compliance, and review of reports required as part of licence conditions, such as retrospective assessments. As part of the audits, an Inspector's role is to assess the systems that an establishment has in place to implement the 3Rs and advise where they are not adequate.

Replacement

ASRU has always found replacement the most difficult of the 3Rs to deal with. ASRU Inspectors are required to have a broad general knowledge of the life sciences and 3Rs issues but are not required to be technical experts in all 3Rs approaches. It would not be possible to maintain up to date knowledge about all available replacement technologies and their suitability across the full range of research areas for which an Inspector reviews licence applications.

Applicants are required to explain what steps they have taken to research alternatives, and whether they have fully considered practicable alternative approaches. They are also asked what *in silico*, *in vitro* or *ex vivo* techniques are used in the project overall and how they integrate with the proposed animal use.

Inspectors assessing PPL applications will look for good answers to these questions but do not necessarily have the expertise to suggest replacements or challenge if the applicant says that replacements that have been considered are either not available or not suitable.

Reduction

Inspectors are not required to be experts in statistics and experimental design. When reviewing a PPL application they review the basic principles of experimental design, but do not carry out a detailed assessment of the proposed statistical methods. They evaluate, as part of an audit, the systems in place at establishments to ensure that correct experimental design and statistical methods are used, and whether local expertise is available.

Licence applicants are required to provide information on various aspects of experimental design, but it is not possible to predict five years ahead exactly what experiments will be done and thus to provide all the information to allow detailed scrutiny of the experimental design. Inspectors look for mentions of randomisation, masking/blinding, the use of appropriate controls, and a credible explanation for the estimated numbers, supported by power calculations if appropriate. They also check licence applications to ensure that any known duplication of procedures is justified. Problems with experimental design, such as lack of masking/blinding or inappropriate experimental units, might have been noticed at site inspections, but this is less likely with ASRU's new ways of working.

Licence applicants must explain how they will ensure any breeding of genetically altered (GA) lines is as efficient as possible and genetic integrity is maintained. Use of best practice in breeding is also reviewed during the audit of establishments. Unusually high numbers of animals culled without being used in experimental procedures may indicate potential problems with colony management.

Refinement

ASRU finds refinement the easiest of the 3Rs to deal with. Licence applicants are asked to explain the choice of animals, models, and methods, why they are the most refined available, and how suffering will be minimised. Inspectors check that any form of animal suffering is justified in a licence application and relevant to the proposed programme of work. Specific aspects of refinement that are explored during licence review are aseptic surgery, the use of non-recovery anaesthesia, and the use of anaesthetics and analgesia. However, it is the NVS's responsibility to advise what anaesthetics or analgesic drugs are most appropriate.

Inspectors look carefully for appropriate humane endpoints and challenge whether experiments could be stopped sooner. The Inspector's role is to assess (as part of audit) the systems that the establishment has in place to ensure the most refined techniques are used and advise where these systems are not adequate. Refinement of housing and husbandry is predominantly assessed during facilities audits.

Does having peer-reviewed funding affect AWERB/ASRU decisions on project licences (or *vice versa*)?

There were varied views on the extent to which the outcome of AWERB review is influenced by whether the research has already been peer-reviewed and funded. Some people said that having peer-reviewed funding in place made AWERBs more confident in the scientific benefits of the proposed work and thus influenced the weighing of harms to the animals used and likely benefits of the proposed research.

Others mentioned that it was rare to see a licence application for which there was not at least some peer-reviewed funding already in place, so it was difficult to identify any influence. People were conscious of the high cost of animal research and felt that if funders were willing to pay it showed that non-animal replacements were not practicable, and that the most appropriate animal models were being used. However, as discussed above, funders may not be explicitly looking at replacement options in their reviews.

Some people commented that where the research had already been peer-reviewed and funded it was difficult for AWERBs to challenge aspects of the plans that they were unhappy with. A few people said that they were aware of specific occasions where pressure had been put on AWERBs to approve licence applications they were unhappy with because the work had already attracted big grants, or that the AWERB felt they were under time pressure related to the availability of grant funding. Others said specifically that they had never seen this happen.

ASRU requires PPL applicants to provide information on how they plan to fund their work. This is to provide assurance that research can be completed, and that benefits will be realised from the use of the animals; peer-reviewed funding also gives some assurance of the quality of the research and that scientific advances will be made. Thus, having peer-reviewed funding influences the harm/benefit analysis Inspectors are required to undertake before granting a PPL.

In contrast, none of the funders thought that having a PPL in place before the grant application was considered influenced the decision about whether using animals was appropriate. However, if a researcher already has the animal model established in their laboratory it does give reviewers greater confidence that they can achieve their objectives for the grant.

What are the barriers to the uptake of 3Rs advances, and what might help to overcome them?

In discussing the reasons for slow uptake of 3Rs advances and any experience interviewees had of people being reluctant to try new methods, some common themes emerged.

The time and cost involved in setting up new techniques in a laboratory, together with a lack of access to expert help, is clearly an issue. People need access to equipment and expert help to enable them to try out new techniques to see if they are suitable for their own research. The laboratories which have developed new techniques do not have the time and resources to help everyone who wants to try them. Researchers may be concerned that if they apply for a grant which involves introducing new methodology their lack of expertise might make their grant application uncompetitive, and that delays in producing data and publications while they get a new technique established will negatively affect their track record and career prospects.

Lack of published studies on how replacement technologies compare to established animal models can be a problem, and academic researchers are concerned that this will prevent their work being accepted for publication. Introducing new replacement methods may be particularly difficult when there is an accepted "gold standard" model in the field.

For researchers developing new treatments, there may also be concerns whether the regulator will accept the evidence to support moving into clinical studies. Many researchers think that they must use an animal model because a paper using only *in vitro* model(s) will not be accepted by the major international journals and editors will ask them to demonstrate their results are valid in an *in vivo* model before a paper can be published.

Where researchers are used to working with a particular model there may be concerns that introducing refinements to experimental protocols will introduce a source of variability or result in a lack of compatibility with earlier data that has already been published.

Many people highlighted the need for better availability of information on advances in all three 'Rs' for researchers, committee members, reviewers and named persons, including information on evaluation and validation of new methods, and signposting of new methods for which the evidence base is strong. It was suggested that specialist scientific societies would be well placed to curate information on replacements and refinements to commonly used models in their field and to challenge the status quo. Sharing of information on approaches that had been tried unsuccessfully would also be extremely useful. The need to better define and resource the role of the NIO was highlighted, in order to help researchers and AWERBs to access information.

Conclusions

Replacement is the area least well covered by existing review processes; the possibility for replacement is best considered at an early stage of the research planning process as AWERBs find it difficult to challenge once funding is in place. AWERBs and ASRU rarely have the detailed scientific expertise to determine whether replacements are available and suitable, so the best strategy for improving this situation would be to ensure that the expert peer review organised by the funders explicitly covers this area.

Improving the availability of information on replacements and how they compare to established animal methods, the ability of NIOs to help researchers fulfil their responsibilities to search for replacements, and the access to expert help and funding to try out new methodologies should help speed uptake of replacement methods.

Review processes scrutinise experimental design and statistical analysis to ensure the numbers of animals used is optimised to obtain robust and reproducible results and avoid the waste of animals that occurs when experiments are under- (or over-) powered.

This is the area where there is the greatest potential for overlap between AWERB, ASRU and funder review and it is important to make the most efficient use of the limited specialist expertise available for reviewing.

More attention should be paid to considering the suitability of new methodologies that allow more data to be obtained from fewer animals as a reduction strategy. Ensuring efficient colony management and GA animal breeding is an important role for the AWERB and is best done at a facility-wide level rather than as part of PPL review by AWERBs and ASRU. This would remove the need for information on breeding strategies to be included in PPL and grant applications.

Refinement is the area that is covered best by AWERB and ASRU PPL reviews and where NACWOs, NVSs and facility staff are most confident to provide challenge. Funders rarely consider this area, except in particularly ethically sensitive situations where they involve the NC3Rs in the review, or where housing, husbandry or animal stress levels may have a particular influence on experimental outcomes.

There is insufficient evidence from this project as to what practices for AWERBs are “particularly effective” (see project aim 2), but practices that some AWERBs or establishments might wish to consider trying are:

- Presentations to the AWERB by people wanting to apply for a new licence before they start drafting their PPL application, to allow a chance for a proper ethical discussion;
- Requiring all applicants to meet the NVS, NACWO, NIO and facility manager to get input at the drafting stage for all licence applications and significant amendments;
- Requiring applicants to attend the AWERB meeting when their PPL application or major amendment to an existing licence is considered;
- Online posting of comments so applicants can think about their response before the AWERB meeting (but this should not replace discussion at the meeting);
- Effective use of subcommittees to ensure the AWERB covers its full remit;
- Review of individual study plans before each study starts, with study plan templates informed by the ARRIVE guidelines;
- Standard housing and husbandry protocols for the establishment prepared by a 3Rs subcommittee, approved by the AWERB and reviewed annually.

Recommendations

1. Funders should make best use of their access to highly specialist scientific peer reviewers to ensure that possibilities for use of replacements or new approaches to obtain more information from fewer animals are identified and implemented where appropriate. This could be facilitated by using more specific questions for reviewers on whether there are available alternatives and/or reduction strategies.

2. Funders could introduce more targeted questions for applicants to elicit information on replacement and reduction, and guidance for applicants on expectations, with the assumption that in most cases[12] optimising refinement will be ensured by ASRU and AWERB oversight.

3. Funders should be prepared to provide additional funding to allow grant holders to explore and validate the use of new alternatives alongside their established models, and to facilitate dissemination of new methods[13] by supporting laboratories which have developed them to provide access to the technology and train others to use it.

4. It should be made clear in a PPL application what parts of the work have already been funded (including date of award and duration) and by whom, so that AWERBs and ASRU are clear what has not. Funders should be willing to share information on whether their expert review has explicitly considered whether replacements are available.

5. Establishments should ensure that their processes allow the use of animals to be challenged early in the research planning process. AWERBs should ask questions about whether/how an applicant has searched for information on possible replacements or reduction strategies. They should expect a clear explanation of what replacements have been considered and why they are not suitable, and whether approaches to get more information from a group of animals have been considered. This could be facilitated by guidance to AWERBs on questions to ask and what should reasonably be expected of applicants.

6. Best practice for induction for AWERB members should include training in the 3Rs and the principles of experimental design. The introduction of audit processes in ASRU's new ways of working provides an opportunity to clarify expectations for training of AWERB members and to confirm via audit that these are being followed. In the longer term the requirement for CPD for all AWERB members should be considered by the sector, in line with the Research Ethics Committees which cover projects involving human participants.

[12] Exceptions might be for specially protected species and/or severe protocols or work to be done in another country.
[13] There are opportunities for partnership funding with the NC3Rs.

7. AWERBs should be clear on the expectations for their role in promoting the 3Rs on a facility-wide basis outside the process of PPL review, including the importance of spending enough time and attention on this part of their role and what constitutes good practice. Areas to cover include refinement of housing and husbandry, efficient colony management and breeding, good experimental design, tissue sharing and sharing of 3Rs advances.

8. The expectations of the NIO role should be set out clearly at each establishment in line with ASPA and LASA/IAT guidance[14]. Establishments must ensure that NIOs have the expertise, time and appropriate resources and training to effectively support researchers, AWERB members and animal facility staff in accessing information on 3Rs advances. They should be well trained in approaches to search for information and have time to support researchers to fulfil their responsibility to look for alternative approaches. ASRU should cover the effectiveness of the NIO role in their audits.

9. To facilitate access to information about 3Rs advances, the NC3Rs, scientific or learned societies and/or funders should convene expert groups to review information on 3Rs advances available in particular scientific areas or for commonly used animal models of disease, to produce authoritative, up-to-date and easily accessible information for researchers, peer reviewers and AWERBs. Funders should ensure that this information is taken into account in their funding decisions.

10. All AWERBs and funder review panels should have access to expertise in statistics and experimental design. Inventive solutions may be necessary to make best use of available expertise for reviewing given the shortage. The NC3Rs Experimental Design Assistant (EDA) should be more widely used in applications; this may require further development to make it more accessible. With the current focus on improving reproducibility across the life sciences, funders and universities should explore means to support development of more experts in statistics and experimental design, both to help and train researchers on the ground and to participate in expert review.

11. ASRU and AWERBs should ensure that information on 3Rs advances obtained from retrospective reviews and retrospective assessments of PPLs is available to the research community, whether via publication or some other means[15].

12. To reduce unnecessary bureaucracy funders can rely on AWERBs and ASRU for checking implementation of refinement and on ASRU to monitor compliance with ASPA (for example, it is not necessary to include this in funder assurance checks or to ask for formal confirmation of licences before grant funds are released). However, it remains important for funders to check that AWERBs have reviewed any animal research that falls outside of the ASPA, such as work taking place overseas.

[14] Guiding Principles for Named Persons | LASA.
[15] For example, a repository that is easily accessible and searchable.

Annex 1: Interviewees

I am very grateful to the following people for contributing to this project by participating in interviews or providing written input.

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Dr Kate Chandler	Animals in Science Regulation Unit
Dr David Coutts	Multiple Sclerosis Society
Lauren Cresser	Pirbright Institute
Andrew Cunningham	University of Sussex
Ngaire Dennison	University of Dundee
Dr Colin Dempsey	Wellcome
Professor David Dexter	Parkinsons UK
Dr Megan Dowie	MRC
Helen Emery	University of Leicester
Dr Anne-Marie Farmer	University of Cambridge
Glyn Fisher	King's College London
Dr Richard Francis	Stroke Association
Maggie Gentry	University of Cambridge
Dr Penny Hawkins	RSPCA
Linda Horan	University of Strathclyde; Animals in Science Committee
Dr Matt Kaiser	Cancer Research UK (CRUK)
Darby Knight	BBSRC
Dr Elliot Lilley	NC3Rs
Professor David Main	Royal Agricultural University; Animals in Science Committee
Dr Jacqui Marshall	CRUK
Stephanie Masefield	BBSRC
Professor Rory McCrimmon	University of Dundee
Dr Kevin Moreton	MRC Laboratory of Molecular Biology
Dr Kathy Murphy	University of Newcastle
Anna Myat	Wellcome
Dr Ivan Pavlov	MRC
Dr Mark Prescott	NC3Rs
Will Reynolds	Animals in Science Regulation Unit; Animals in Science Policy and Coordination Unit
Professor Emma Robinson	University of Bristol
Dr Sally Robinson	AWERB Chair CRUK Manchester Institute; AWERB Chair University of Bristol; Animals in Science Committee
Dr Jo Roe	University of Bristol
Dr Kathryn Ryder	Northern Ireland ASPA Inspector
Dr Danielle Sagar	BBSRC
Professor Owen Sansom	CRUK Beatson Institute, University of Glasgow
Dr Subreena Simrick	British Heart Foundation
Dr Geraldine Taylor	Pirbright Institute
Professor Richard Thomas	University of Birmingham
Professor Andrew Trafford	University of Manchester
Dr Martin Vinnell	University of Cambridge
Dr Sara Wells	Mary Lyon Centre, MRC Harwell

Annex 2: Glossary

AMRC	Association of Medical Research Charities
ASPA	Animals (Scientific Procedures) Act (1986)
ASRU	Animals in Science Regulation Unit
AWERB	Animal Welfare and Ethical Review Body
BBSRC	Biotechnology and Biological Sciences Research Council
CRUK	Cancer Research UK
EDA	NC3Rs Experimental Design Assistant
ELH	Establishment Licence Holder
GA	Genetically Altered
LASA	Laboratory Animal Science Association
IAT	Institute of Animal Technology
MRC	Medical Research Council
NACWO	Named Animal Care and Welfare Officer
NC3Rs	National Centre for the Replacement, Refinement and Reduction of Animals in Research
NIO	Named Information Officer
NVS	Named Veterinary Surgeon
PPL	Project Licence
RSPCA	Royal Society for the Prevention of Cruelty to Animals
3Rs	Replacement, Reduction and Refinement