Internationalisation and Harmonisation of Laboratory Animal Care and Use Issues

Proceedings of the 9th FELASA Symposium

4-17 June 2004, Nantes, France

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"Wherever possible, specialists should not be segregated in separate laboratories. The aim should rather be to assemble as many different kinds as possible under one roof."

Russell & Burch (1959)

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Laboratory Animals – the journal

Laboratory Animals has been at the forefront of laboratory animal science for 30 years and is now the official journal of FELASA, as well as of the German (GV-SOLAS), Israeli (ILAF), British (LASA), Dutch (NVP), Spanish (SECAL) and Swiss (SGV) national laboratory animal science associations. The journal publishes papers dealing with all aspects of the use of animals in biomedical research, including:

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The Editorial Board of Laboratory Animals wish to give especial encouragement to appears describing work which involves a reduction in the number of animals that need to be used, or which replaces animals with in vitro alternatives; or which represents a significant refinement in methodology, leading to improvements in the welfare or well-being of the animals used. There is full on-line access available at no extra charge to subscribers.

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The History of FELASA – Birth and Growth 1978-2004

Guy Mahouy, AFSTAL Honorary President

The Federation of European Laboratory Animal Science Associations (FELASA) is composed, at present, of 12 independent European national and regional laboratory animal science associations. It can speak for laboratory animal scientists and technologists in at least 20 countries: Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Latvia, Lithuania, the Netherlands, Norway, Spain, Sweden, Switzerland and the United Kingdom. A Board consisting of Officers and representatives of its constituent associations manages this Federation. Established in 1978, it has significantly grown over the years and is now recognised both at European and international levels.

After 27 years of existence, it seems worthwhile to remember why and how such a structure was implemented and what were the initial objectives and mission statements of FELASA.

In the mid-seventies, there was a trend in Europe to develop much closer communication links between the different European laboratory animal science associations. Although nothing concrete was done at that time, individuals like Karl-Johan Öbrink, Lars Wass (from Scand-LAS), John Bleby, Philip O'Donoghue, Peter Eaton, Malcolm Gamble, Roy Ward (from LASA), Klaus Bonath, H Bruhin, Anthony Ellery (from GV-SOLAS) and many others were discussing what should be the best way to initiate such a cooperation, particularly the organization of joint scientific meetings.

Accordingly, the "Preliminary" meeting was the first joint GV-SOLAS/LASA/Scand-LAS meeting on "The Laboratory Rat and Biological Variation" held on 26-28 June 1978 at Churchill College, Cambridge, UK. Representatives of the three associations met and it was suggested that a joint scientific meeting should be organized every three years by one of these associations in turn and that the FELASA Presidium should be run by the association arranging the next meeting. This suggestion was formally confirmed at a meeting in Utrecht on 21 August 1979. Therefore, as the German society proposed to host the symposium in 1981, the FELASA Presidium from 1978 to 1981 was filled by the GV-SOLAS President and Secretary. This was the foundation meeting of FELASA by these three European associations. The Federation was soon joined by the Dutch "NVP" and the French "SFEA", and then progressively by others European associations.

The first FELASA Symposium was organized in Düsseldorf, Germany, by GV-SOLAS in 1981 and at the first General Assembly the constitution of FELASA was adopted. This Symposium was followed by others on a three years basis, in Malmö, Sweden (Scand-LAS, 1984), in Amsterdam, the Netherlands (NVP, 1987), in Lyon, France (SFEA now known as AFSTAL, 1990), in Brighton, UK (LASA, 1993), in Basel, Switzerland (GV-SOLAS, 1996), in Palma de Mallorca, Spain (SECAL, 1999), in Aachen, Germany (GV-SOLAS, 2002), in Nantes, France (AFSTAL-ex SFEA, 2004).

The organization of these triennial symposia has been the principal activity of FELASA from the outset until 1987, and proved of good service to European laboratory animal science. However, in between these symposia, very little happened and it was clear that some reorganisation had to be considered to address emerging issues.

Informal discussions about the role of FELASA were held among officers of constituent associations at the third FELASA Symposium in June 1987 in Amsterdam (NVP). It was generally agreed that the Federation should become more active and a meeting was therefore convened in October to see how this could be done.

This important joint meeting was organized by Karl-Johan Öbrink and Lars Wass (Scand-LAS) on 17-19 October 1987 at Odalgärden, near Uppsala, Sweden. The aim of this conference was to reflect on the future of FELASA. All constituent associations were asked to send representatives, and a number of individuals were invited to take part in the discussion. It was a very constructive meeting and the following proposals were made. :

1) To ensure the good functioning and the implementation of new activities for the Federation, it was essential to revise and modify the initial FELASA constitution. One of the reasons was that the President and the Secretary of the constituent association hosting the triennial conference were automatically President and Secretary of the Federation. It was clear that these officers were far too busy arranging the next Symposium and did not have the capacity to address other issues. Accordingly, the new constitution should include new organisational structures, which should be revised and accepted by all the constituent associations. The Federation would have a Board of Management in which each constituent association would have two members. The Board would annually elect three officers who, together with the President, would be directly responsible for the administrative and organisational work. The most obvious change should be that FELASA would have its own officers elected by the Board from amongst its members, with duties and term of service that were not tied to the triennial symposia.
2) The Federation should establish a policy group in order to identify topics for further study.
3) It was seen as essential for FELASA to secure recognition in Europe - through both the Council of Europe and the European Communities Commission - as the authority to be consulted on all matters relating to laboratory animal science, with consequent strong links to Strasbourg and Brussels.
4) A specific task of considerable importance should be the production of appropriate education and training programmes for all those involved in the laboratory animal field. A FELASA Working Group on Education should be created to develop

training programmes for the different categories of animal users: animal caretakers, animal technicians, scientists actually using laboratory animals and laboratory animal science specialists. The first step should concern the education and training of competent authorised persons and, particularly, scientists using laboratory animals. A common standard would facilitate the professional mobility of scientific workers within Europe.

5) FELASA should also establish a Working Group on Animal Health to encourage uniformity in assessing animals and contributing to standardisation and high standards of science and animal welfare.
6) It should also establish and maintain appropriate links with international or other bodies concerned with laboratory animal science.

All resolutions from the meeting were sent to the respective associations for agreement and ratification. This was considered fundamental to the future development and increased recognition of FELASA. The revised constitution was also circulated before the FELASA Symposium in Lyon, France (1990) and ratified. An official Board of Management with an Executive Committee and its own officers was established.

This revised structure has significantly aided the Federation in its work. The national member or regional bodies arrange the triennial meetings. While this important work is under way and occupying much of the host organisation's resources and attention, FELASA Officers and Governing Board are free to concentrate on wider and longer term issues.

In pursuing its aim of achieving European recognition, we have had to recognise that there are differences in structure and working practice between the Council of Europe (CoE) in Strasbourg and the European Communities Commission (part of the European Union, EU) in Brussels. In November 1991, FELASA was granted observer status for the sector of laboratory animals. The first success was with the CoE, which welcomed FELASA's recommendations for the education and training of all those authorised to raise, maintain and use laboratory animals. The CoE pressed the Federation to extend its advice and, when satisfied, adopted the recommendations as official CoE policy and formally thanked FELASA for its work. Once CoE policy, the FELASA proposals were considered in Brussels and adopted, more or less unchanged, as EU policy.

FELASA also seeks to play its part in wider issues by establishing fraternal relations with laboratory animal science associations outside Europe and by collaboration with international bodies (the 7th FELASA Symposium was a joint meeting with the International Council for Laboratory Animal Science-ICLAS) and other organisations with shared interests (on 17-18 December 1996 an European Congress on "The Ethics of Animal Experimentation" was organised in Brussels, Belgium, by the European Biomedical Research Association-EBRA- in conjunction with FELASA, with its proceedings edited by P. N. O'Donoghue and published by EBRA, London, UK, in 1998).

There are many other issues on which FELASA advises and you will find below a table of FELASA recommendations, publications and policy documents. Its strength in such matters is largely because it can speak for such a wide cross section of European laboratory animal scientists. When it has settled its policy by debate among its member associations, it can promote that policy with considerable authority. It is the European body most suitable to define, periodically review and to promote the best possible practise in all aspects of laboratory animal science.

FELASA International Symposia

- 1st Symposium: "First Scientific Meeting of the Federation of European Laboratory **A**nimal Science Associations (FELASA)", Düsseldorf, Germany, 2-4 June 1981.
Organised by GV-SOLAS.
Only Programme and Abstracts available.

- 2nd Symposium: "Second FELASA Symposium", Malmö, Sweden, 16-21 June 1984.
Organised by Scand-LAS.
Abstracts published in *Z. für Versuchstierkunde* (1985), **27,** 57-119.

- 3rd Symposium: " New Developments in Biosciences: Their Implications for Laboratory Animal Science", Amsterdam, The Netherlands, 1-5 June 1987. Organised by NVP.
Proceedings edited by A.C. Beynen and H.A. Solleveid.
Published by Martinus Nijhoff, 1988

- 4th Symposium: " Man and the Laboratory Animal: Perspectives for 1990", Lyon, France, 10-15 June 1990
Organised by SFEA (now known as AFSTAL).
Published by Fondation Marcel Mérieux, Lyon, 1990

- 5th Symposium: "Welfare and Science", Brighton, UK, 8-11 June 1993
Organized by LASA.
Proceedings edited by J. Bunyan.
Published by The Royal Society of Medicine Press Ltd, London, UK, 1994

- 6th Symposium: "Harmonization of Laboratory Animal Husbandry", Basel, Switzerland, 19- 21 June 1996.
Organized by SGV.
Proceedings edited by P.N. O'Donoghue.
Published by The Royal Society of Medicine Press Ltd, London, UK, 1997

-7th Symposium: "Animal Research and Welfare: A Partnership", FELASA-ICLAS Joint Meeting,
Palma de Mallorca, 26-28 May 1999. Organized by SECAL.
Proceedings edited by J.A. Tur-Mari and J.M. Orellana-Muriana
Published by Laboratory Animals Ltd, London, UK, 2000

- 8th Symposium: "Laboratory Animal Science – Basis and Strategy for Animal Experimentation",
Aachen, Germany, 17-20 June 2002.
Organized by GV-SOLAS.
Proceedings edited by J. -L. Guenet and C. Herweg
Published by Laboratory Animals Ltd, London, UK, 2003

- 9th Symposium: "Internationalization and Harmonisation in Laboratory Animal Care and Use Issues",
Nantes, France, 14-17 June 2004. Organized by AFSTAL.
Proceedings edited by M. Gamble and S. Millington
Published by Laboratory Animals Ltd, London, UK, 2005

FELASA recommendations, publications and policy documents

FELASA recommendations and publications

Education and training

- FELASA recommendations on the education and training of persons working with laboratory animals: Category A and C
Laboratory Animals (1995) **29**: 121-131

- FELASA recommendations for the education and training of persons carrying out animal experiments: Category B
Laboratory Animals (2000) **34**: 229-235

- FELASA guidelines for the education of specialists in laboratory animal science (Category D)
Laboratory Animals (1999) **33:** 1-15

Health monitoring

- Health monitoring of breeding colonies and experimental units of cats, dogs and pigs
Laboratory Animals (1998) **32**, 1-17

- Health monitoring of non-human primate colonies

Laboratory Animals (1999) **33** (Suppl.1), 51**:**3-51:18

- FELASA recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units
Laboratory Animals (2002) **36:** 20-42

- FELASA recommendations for the health monitoring of experimental units of calves, sheep and goats
Laboratory Animals (2000) **34**: 329-350

Nutrition
- FELASA-Quick reference paper on laboratory animals feeding and nutrition

Others
- FELASA guidance paper for the accreditation of laboratory animals diagnostic laboratories
Laboratory Animals (1999) **33:** (Suppl.1), 51:19-51:38

- FELASA statement on nonhuman primates

- Pain and distress in laboratory rodents and lagomorphs
Laboratory Animals (1994) **28**: 97-112)

- Sanitary aspects of handling nonhuman primates during transport
Laboratory Animals (1997) **31**: 298-302

FELASA members

AFSTAL	Association Française des Sciences et Techniques de l'Animal de Laboratoire
AISAL	Associazione Italiana per Scienze degli Animali da Laboratorio
Balt-LASA	Baltic Laboratory Animal Science Association
BCLAS	Belgian Council for Laboratory Animal Science
CLASA	Czech Laboratory Animal Science Association
GV-SOLAS	Gesellschaft für Versuchstierkunde - German Society for Laboratory Animal Science
HSBLAS	Hellenic Society of Biomedical and Laboratory Animal Science
LASA	Laboratory Animal Science Association (United Kingdom)
NVP	Nederlandse Vereniging voor Proefdierkunde
Scand-LAS	Scandinavian Society for Laboratory Animal Science
SECAL	Sociedad Española para las Ciencias del Animal de Laboratorio
SGV	Schweizerische Gesellschaft für Versuchstierkunde

Where written papers were not submitted abstracts only have been inserted

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How could Harmonisation help in Implementing Principles?

The contribution and influence of FELASA in legislative reform in Europe and elsewhere

Derek Forbes, President, FELASA, 25 Shaftesbury Ave., London W1D 7EG, UK

FELASA, being one of several organisations having pan-european recognition has been represented at meetings and working parties involved in the revision of legislation, which regulates the use of experimental animals. For some years now the Appendix A of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS 123) has been under revision. The revision has been comprehensive and species that were not included in the original appendix have been added so that all animals now have similar protection. Throughout the process representatives of FELASA have been members of all the species-specific working groups advising the national parties, and in addition, it has had observer status at all the working party meetings. This process is now nearing its completion and FELASA is planning to produce a concise booklet (The Euro guide) summarising the key points, to be used as a working manual. More recently, the European Commission has initiated the promised revision of the Directive 86/609. Four Technical Expert Working Groups were set up and experts from within FELASA were included within each group. Once again, the revision is intended to be comprehensive. The topics the groups considered were: Scope, Authorisation, Cost-Benefit and Ethics. Probably as a consequence of some of the individuals concerned, some were invited as speakers to an ILAR meeting in Washington. This was an International Workshop on Development of Science-Based Guidelines for Laboratory Animal Care. These developments to harmonise standards in Europe and in due course, possibly internationally, should do much to avoid replication of studies and significantly reduce the number of animals used. Equally as important is the process by which the revisions have been conducted. The involvement of organisations covering the whole spectrum of animal use, care and welfare is surely the best way to gain public acceptance of the continuing need to use animals in research.

Harmonisation and 3Rs Alternatives

Timo Nevalainen, National Laboratory Animal Center, University of Kuopio and Veterinary Faculty, University of Helsinki, Finland

Summary

Harmonization means agreement of action, adaptation or effect. The European Directive and Convention are such agreements, which are supplemented with further regulations like those in the Appendices. The relation of these agreements to 3Rs becomes clear in the wording of the EU Directive (86/609/EEC): An experiment shall not be performed on an animal, if another scientifically satisfactory method of obtaining the result sought, is reasonably and practicably available (Replacement). Furthermore, the European Commission and the EU Member States must actively encourage and support the development, validation and acceptance of methods which could reduce (Reduction), refine (Refinement) or replace the use of laboratory animals. This statement clearly calls for research on the 3Rs as the primary tool, which should show the method to be valid or effective for the purpose. The resulting regulations should be considered as minimum standards, as is obvious in such areas as space allocations. In addition to space regulations, the revised Appendix A aims at furthering Refinement through group housing and enrichment requirements. While Refinement and Replacement aims can mostly be connected to research data, Reduction alternatives suffer from lack of research on which to base regulations. In addition to the regulatory documents, there is a multitude of guidelines, like those of FELASA, which aim at excellence beyond the minimum standards. The latter can be updated with much less procedure than regulatory ones, which is sometimes necessary when new scientific results become available. The key question is where the balance between binding regulation and voluntary guidelines should be set for effective, but flexible sets of guiding documents and consequently best possible outcome for the 3Rs.

Key words: Replacement, refinement, reduction, alternatives, harmonization

The use and welfare of laboratory animals in research are issues of major concern in the modern society. Both public requests to promote the animal welfare and continuously increasing demands on quality and scientific validity of biomedical research make the issues urgent and complex. As a result, legislation and recommendations for the protection of laboratory animals are now under revision both at the European and national levels.

Many of the controversies and concerns over the use of laboratory animals are associated with medical research. This may have a historical basis. During the 1800's French physiologists Francois Magendie and Claude Bernard and their German students Rudolf Buchheim and Oswald Schmiedeberg discovered many physiological mechanisms. At that time there were no anaesthetics available, and studies had to be carried out without them. The use of anesthetics became known at about 1850. The British were keen on the proper treatment of animals even then, and their physiological research blossomed during the 1870's.

Alternatives

Sometimes good ideas have been presented prematurely, and hence partly forgotten. Agood example of this is the five principles put forward by Marshall Hall already 1831. His first principle says that no experiment should be done if the same information can be gained with simple observation. The second principle calls for clear aim of the study, and necessity to make sure that the aim can be achieved. The third principle emphasizes avoiding repetition unless there is reason to suspect errors or need to confirm the results. The fourth principle states that an experiment should be executed with the least possible suffering to the animal. The fifth principle requires that each study should be done in such an environment that proper observation and valid results are secured and that there should be no need for repetition (Paton 1984). All these principles are still valid and have clear connection to the 3Rs alternatives, as we know them today.

More than hundred years elapsed before the current definitions of alternatives were introduced.

Harmonisation

Harmonization means agreement of action, adaptation or effect. Within Europe harmonization is usually understood as agreement between member countries of the Council of Europe (CoE) or the European Union (EU). The Directive and the Convention as such are agreements, which are supplemented with further regulations like those in the Appendices. The relation of these agreements to 3Rs becomes clear in the wording of the Council Directive (86/609/EEC): An experiment shall not be performed on an animal, if another scientifically satisfactory method of obtaining the result sought, is reasonably and practicably available (Replacement). Furthermore, the Directive states that the EU Member States must actively encourage and support the development, validation and acceptance of methods which could *replace, reduce and refine* the use of laboratory animals (3 Rs). The same is true for the policy paper of the European Science Foundation (ESF 2001).

The Ethical Rules for the 6th Framework Program (FP6) proposals reiterate the application of the 3 Rs principles and entail a description of the procedures adopted to ensure that the amount of suffering imposed to the animals is minimized and their welfare is guaranteed as far as possible (*e.g*. through improvements in experimental technique, application of humane end-points, environmental enrichment, *etc*.). According to the Ethical Rules for the FP6 proposals, applicants should provide a summary of the main adverse effects for the animals, including those due to methods of husbandry and supply of the animals as well as the harmful effects of the scientific procedures themselves.

The expert working groups nominated by CoE to propose the revision of ETS123 Appendix A (Housing and Care of Laboratory Animals) have noted that, although many of the resulting recommendations could be backed with research

data, still in many instances they had to be based on best practice. This clearly demonstrates that more research is needed in the the Two Rs (Refinement and Reduction) to yield evidence-based results, which could lead to valid guidelines and recommendations.

The Report on the Directive 86/609 (2001/2259(INI)) has stated that the research proposals using animals must clearly substantiate and justify the purpose and demonstrate that the experiments are aimed to promote animal or human health. The Report states more specifically that an ethical and animal-welfare assessment must be carried setting limits to the level of suffering and distress to which the animals may be subjected. Furthermore, the report called for a cost or harm-benefit analysis as an integral part of any ethical review, as did earlier ESF policy statement: 'Use of Animals in Research' (ESF 2001). The Ethical Rules for FP6 require applicants to 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.

How to further harmonization and the alternatives

All the statements cited above clearly call for research on the 3 Rs as the primary tool, which should show the method to be valid or effective for the purpose. The resulting regulations should be considered as minimum standards, as is obvious *e.g.,* in space allocations of the present and revised Appendix A of CoE. In addition to space regulations, the revised Appendix A aims at furthering Refinement through group housing and enrichment requirements. While Refinement and Replacement aims can mostly be connected to research data, Reduction alternative suffers from lack of research to base regulations on.

Until now the Replacement alternative has received far more attention and EU funding opportunities than the other two Rs – reduction and refinement. All the 3Rs should deserve equal weight and support in research funding. Replacement is not always possible and sometimes not even desirable. Therefore, more research on the remaining Two Rs should be encouraged in order to improve welfare of the animals still being used, leading to better quality animals and to a reduction of the numbers used. Moreover, it may be that better science evolves from the application of the Two Rs.

Any Refinement and Reduction alternative should be scientifically proven to have beneficial effects on the animals and not to interfere with the results of the study at the same time. These criteria should be considered as key requisites in the practicability assessment of the Two Rs. This is in line with the Council of Europe (CoE ETS 123, 3.5.2004) statement that consideration should be given to the potential impact of the type of accommodation, and that of the environmental and social enrichment programmes, on the outcome of scientific studies, in order to avoid the generation of invalid scientific data and consequential animal wastage.

The application of the Two Rs will also be instrumental when performing the harm-benefit analysis. They should be regarded as means to either increase the benefits or decrease the harm to the animals of a research project as shown in the outcome of the Nordic Forum on ethical evaluation of animal experiments (Voipio *et al*. 2004). This is particularly true with GM-animals used in increasing numbers in modern biomedical research.

European research potential

Any considerable funding to research on the Two Rs is likely to generate new knowledge enabling better welfare for fewer animals in research and consequently ease the concerns of the society. It can also be foreseen that this very same research, if carefully planned and executed, is crucial in avoiding practices and procedures compromising the scientific validity of science. While regulations represent minimum standards based on evidence-based data, the research on the Two Rs should aim further, *i.e.* at excellence above the minimum standards. In essence, the two Rs can be regarded as essential elements of the harm-benefit analysis.

A higher focus on the two Rs will secure that studies are always executed on a high quality level, and that unnecessary duplication is avoided. This supports best return on investments, both in the short term - and in the long-term. Because of the definition of alternatives as furtherance of one or more of the 3 Rs, indeed every scientist using animals can and should actively seek implementation of one or more of the alternatives. There is a clear need to study - and whenever possible implement - the 3Rs. In this context it has to be emphasized that animals do have intrinsic, not only instrumental value.

The EU report (2001/2259(INI)) that started the revision of the Directive reads: An ethical and animal-welfare assessment must be carried out setting limits to the level of stress to which the animals may be subjected. This reflects the trend to set cut off value to compromised animal welfare, thus typically a Refinement aim. The same report called also for a cost/benefit analysis and mandatory training guidelines for all competence categories, both typically aiming at new and higher minimum standards in order to further all the 3Rs.

The document 'Science and technology, the Key to Europe's future - Guidelines for future European Union policy to support research' states that the Commission has made strengthening European research a major objective…is proposing to increase the European Union's research budget….the budget should be doubled (EU 2004). If this is to happen, it will inevitably mean more laboratory animals used in basic research, and acute and urgent need for funding of studies on how best to apply the Two Rs methods.

European added value

Investing in research of the 3Rs alternatives at the same pace with funding of basic research enables Europe to maintain or even increase the lead it has. All research carried out should simply be ethically sustainable and scientifically valid. Yet, this is not simple neither straightforward, and it can only be achieved through tailor made, considerable funding granted on a competitive basis. Excellent education and research in the 3 R's will contribute to social benefits, *i.e.* improved public understanding and acceptance of the way laboratory animals are used. Economic benefits result from the avoidance of unnecessary duplication and executing studies correctly and at a high quality level from the start.

The revision of the Directive (86/609/EEC) is anticipated to require a more detailed and harmonized ethical evaluation of animal experiments throughout Europe. It is also obvious that the system to be established builds on harm-benefit analysis. In this analysis the likely benefits of the study are weighed against the harms – *i.e.* pain, suffering and distress - to the animal. It can be foreseen that both commodities to be weighed have to be broken down to smaller elements in order

to weigh or attach an ethical value judgment to each of them. Then these elements can be used in the overall assessment of an animal study. What is perhaps even more important is improving all areas of concern, identified in the analysis, so that both animal welfare and good science are promoted.

The welfare and number of animals used can be regarded as essential elements in assessment of costs/harms, incurred to the animals in the study. The Two Rs methods represent some of the means, which should be used either to decrease the costs and in some cases to improve the benefits. This approach can only be successful if tackled by a multidisciplinary team, *i.e.* by both the study groups themselves and laboratory animal scientists. Involvement of both parties is necessary for attainment of critical mass, a prerequisite to large scale outcome. And the Two Rs means will be creditable only if they have proven 'efficacy' to animal welfare and proven 'safety' to the study.

European consortia and European funding are needed to coordinate national research activities and studies on the 3 Rs, to attain European added value through implementation of the 3 Rs methods and to show that the European research community practices good ethics in their daily work. By high quality research, improving animal welfare and avoiding unnecessary duplication of animal studies, a valuable contribution to sustainable economic growth is attained within the EU.

Guidelines and recommendation

In addition to the regulatory documents, there is a multitude of voluntary guidelines, like those of FELASA, which aim at excellence beyond the minimum standards. Good examples of FELASA guidelines are those on education and training and health monitoring (FELASA 1995-2002). The latter can be renewed with much lighter procedure than regulatory ones, which is sometimes a necessity when new scientific results become available. And it also can be subjected to harmonization, which indeed is about to start as an international mission.

Recently established COST Action 'Laboratory Animal Science and Welfare' is designed to look for answers to the concerns of both the public and the scientists. The approach chosen is to increase knowledge necessary for both ethically sustainable and scientifically valid use of laboratory animals in research. These two aims are not only possible, but indeed a necessity. The Action serves as an interaction podium and idea generator for scientists and civil servants and paves the way for European research consortia. Furthermore, it aims at the production of research results and collection of technical data based on scientific studies, and ultimately seeks tools needed for real life implementation. Delivery of the processed data is done through harmonizing of training of persons working with animals and as guidelines and recommendations, which should go beyond regulatory minimum standards (COST B24 MoU 2004).

Concluding remarks

The key question is where the balance between binding regulation and voluntary guidelines should be set for an effective, but flexible set of guiding documents and for best possible outcome of the 3Rs. Both the regulatory bodies and the research community have a definite need for evidence based data. This data can only be produced with considerable tailor made funding for all the 3Rs, and consequent recruitment of critical masses of scientists to do the research. And in cases, where no replacement alternatives exist, the animals cannot be neglected, but substantially more Two Rs alternatives must be made available. This is an absolute necessity for both good animal welfare and good quality science.

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European Science Foundation (2001). Use of animals in research.

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The Value of Collaborative Projects in implementing the three 3Rs in Toxicology

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1 Introduction

The dog is the most frequently used non-rodent species in the safety assessment process of new medicines and its value was demonstrated in the review carried out by the International Life Sciences Institute (Olson, *et al.,* 2000). However, other reviews have concluded that it may be possible to achieve a reduction in their use without compromising human safety (Broadhead, *et al*., 2000). A Steering Group representing twelve European pharmaceutical companies and two animal welfare organisations were established in 2000 with the aim of recommending and, where possible, putting into practice scientifically valid and feasible approaches to minimise dog use. The Group has identified many potential approaches and prioritised them for further analysis (Smith, *et al*., 2002). This publication gives an overview of the project together with the learning points that have arisen during the four years that the Group has been collaborating. The project is working on study and project designs that are both within and outside the regulatory framework and this difference in freedom to operate is illustrated in the approaches taken.

2 Project objective

The aim of this initiative is:

- To recommend and, where possible, put into practice, scientifically valid and feasible approaches to minimise dog use in pharmaceutical safety evaluation.
- The initiative will focus on those approaches that will not compromise human safety or the scientific quality of pharmaceutical safety evaluation, or increase the use of other non-rodent species.

3 Potential approaches to minimize dog use

The Steering Group's review of study designs and working practices identified a plethora of potential opportunities to minimize dog use. To focus its effort, the Group prioritized them according to the impact on the number of animals used, the impact on the welfare of the remaining animals, the potential for industry's acceptance of the scientific approach, the potential for regulators' acceptance of the validated approach, and the time/cost of evaluation or implementation.

After prioritisation, the opportunities were categorised into three areas:

- Best practice in study design (Refinement/Reduction)
- Industrial co-operation/data sharing (Reduction)
- Assessing need for particular studies (Replacement)

4 Achieving Best Practice in Study Design

The approaches that have been taken forward for further evaluation are:

- Group sizes for repeat-dose studies
- Appropriate preliminary studies
- Use of single sex studies
- The need for recovery groups
- The use of control animals
- Overall programme design

This publication discusses the first two approaches, group sizes for repeat dose studies and appropriate preliminary studies, to illustrate the value of the collaborative project. All of this work is currently awaiting publication elsewhere.

4.1 Group Sizes for repeat dose studies

Analysis of dose group sizes from twelve European pharmaceutical companies is shown in Table 1:

Table 1. Analysis of twelve European pharmaceutical companies

	Dose Group Sizes No.of dogs/sex/group			
Duration of study	1 m	3 m	6 m	9/12 m
Main study				
Norm	3	3	4	4
Range	2-6	3-6	3-6	3-6
Recovery				
Norm	2	2	2	2
Range	0-2	0-2	0-2	0-2

Although data revealed that the majority of companies were using group sizes consistent with regulatory guidelines, the opportunity for harmonising, with consequent reduction in numbers of animals, is apparent. Sharing of best practice may also result in rationalising the use of recovery (off-dose) animals. When this data was shared with the Steering

Group the majority of companies using the larger group sizes reported that they had modified their study plans accordingly.

The two learning points from this early part of the project can be summarised as:

- Despite the outputs from the International Conference on Harmonisation (ICH) do not assume minimum numbers being used (CPMP/ICH 2000)
- Do not underestimate the power of sharing information to influence change.

4.2 Appropriate preliminary studies

Preliminary dog studies are carried out before the pivotal regulatory study and these are often described as maximum tolerated dose (MTD) or dose/range finding (DRF) studies. In an attempt to optimise the design of such studies a questionnaire was used to elicit the designs currently in use in the twelve pharmaceutical companies. Surprisingly, 15 different designs were identified, reflecting the uses to which these studies could be put. There were variations in many aspects of the design, which lead to a wide variation in the number of animals being used. Before an optimised design could be recommended it was important to identify the primary and secondary purposes of such studies and the consensus is summarised in Table 2.

Having defined the primary purpose of MTD/RDF studies, the Steering Group went on to assess how well current designs met that purpose by analysis of 100 data sets from participating establishments. A questionnaire was used to examine the design of the preliminary studies and the outcome of the regulatory 14 day/1 month study in terms of its success or failure based on the selection of the high dose level. It was judged a success if target organ toxicity was elected or, failing that, if the dose was the maximum technically feasible. The MTD/DRF study was judged a failure if additional animals or an additional dose group had to be used in the 14 day/1 month study or if this study had to be repeated due to inappropriate dose selection.

Table 2. Purpose of MTD/DRF Studies

Primary purpose:	Dose selection (high dose) for pivotal/repeat dose study
Secondary purposes:	Detection of serious toxicity to confirm candidate drug selection.
	Estimation of compound requirements
:	Confirm species selection
:	Obtain toxicokinetic data
:	

An analysis of the results from 101 pivotal 14 day/1 month studies is shown in Figure 1.

The data show that preliminary dose-setting studies that involved the use of up to four dogs are as likely to be successful in predicting appropriate dose levels for the subsequent 14 day/1 month study as are those studies that involved substantially more.

Having taken in to account the primary objective of the preliminary MTD/DRF studies, and after consideration of the information generated from the review of previous studies, the Steering Group proposed a basic design involving an escalating dose phase with one male and one female dog dosed to the MTD, followed by repeat dosing (of >4 days duration) in one male and one female naïve animal at the MTD, with additionally one male and one female non-naïve animal at the same dose or a lower dose. On occasions when animals from the escalating dose phase cannot be re-used in the repeat dose phase (e.g. if the MTD is exceeded), a further two animals would be required (total six animals).

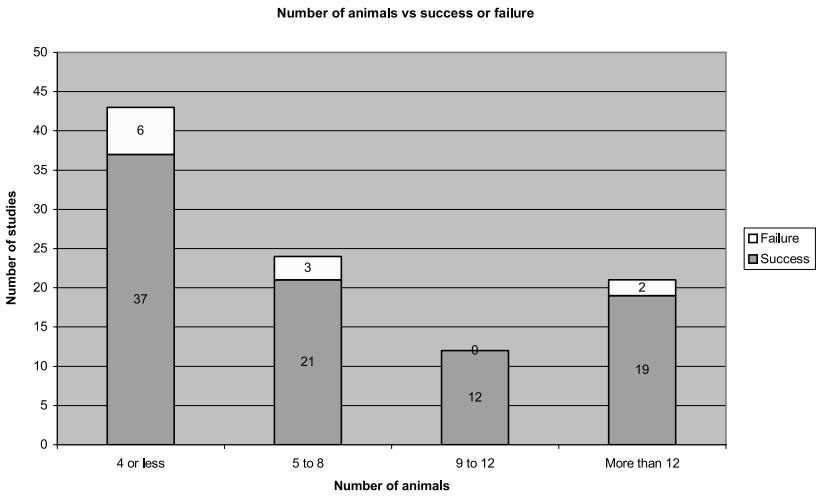


Figure 1 Analysis of the outcome of 101 pivotal dog studies in Relationship to the number of animals used in preliminary studies

An alternative design, which has also been shown to be successful, uses a repeat dose phase with three dose levels and one male and one female per group.

Adoption of these recommended designs globally is expected to lead to a significant reduction in animals. The two learning points from this part of the project are:

- The objectives of a study need to be periodically reviewed to ensure 'need to know' rather than 'nice to know' attributes are included (design creep).
- Do not underestimate the power of sharing scientific data to influence change.

5 Industrial co-operation/ data sharing

The first part of the project demonstrated the value of industrial co-operation, particularly when sharing information. The second part of the project, which has just commenced, will build on this success and tackle the thorny issue of data sharing in a formal and more open way. The aim is to build a database of non-active ingredients of formulations used in preclinical toxicology i.e. a vehicle database.

A vehicle database would contain qualitative and quantitative findings of all vehicles, excipients, solvents and preservatives used in the preparation of dosing formulations and would be "owned" by the industry. Although repetition of studies is rare, there are occasions when vehicles are being used either for the first time or by a different route of administration. Data may not be in the public domain, and sharing of toxicity profiles would avoid the need for investigation/MTD/DRF studies to precede regulatory studies.

This part of the project has received interest from the EU Commission and it is proposed to take this initiative further as a COST proposal to assess the feasibility of such data sharing in Europe. It is likely that both the chemical and pharmaceutical industries will work together on this project.

6 Assessing the need for particular studies

This third part of the project is probably the most challenging. It aims to eliminate the need for terminal three and six month dog studies. If successful it would have a significant effect on animal numbers.

After the one month study, the aim would be to conduct a single study of nine to 12 months duration, which would provide interim data at three and/or six months to allow progression of clinical trials. Necropsies would not be performed at these time points, and the study would rely on biomarkers of toxicity, as in clinical trials.

Currently, it may not be possible to achieve this aim; however, as technology develops, we must be in a position to capitalise on it. To do so, it is necessary to identify toxicities that occur after one month but before nine to 12 months and to assess the potential to detect each case by other means. A database, not unlike that of the International Life Sciences Institute project, would be established to gather such information; and over the same period, a number of the new technologies would be assessed for their ability to detect effects in long-term ongoing studies (e.g. the utility of metabonomics and genomics in the dog - although most work is focussed on the rat). Generation of additional data would also be required to assess how many times an early-initiated study may be aborted because group sizes for the long-term studies are larger than those for the three month studues. Of course, on the positive side, the power of such a single study with increased group size would be increased - an issue frequently raised by regulators such as the US Food and Drug Administration (FDA).

Despite the difficulty envisaged with this part of the project, the Steering Group were given more confidence to proceed following the presentation by David Jacobson-Kram (Associate Director for Pharm/Tox in the Office of New Drugs at CDER, FDA) at a DruSafe meeting in July 2003 when he shared the FDA's short, medium and long-term vision:

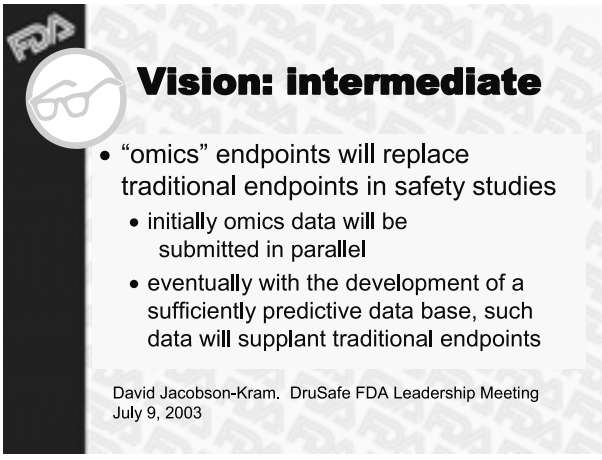
Ultimately, the success of this part of the project will rely upon the international regulatory bodies to accept such an approach. It is envisaged that, after initial contact with the European regulatory agency (through the Committee for Proprietary Medicinal Products, CPMP), the International Committee on Harmonisation, ICH, would need to address MS3 (the timing on non-clinical studies) and S4A (the duration of repeat dose studies in non-rodent).

7 General learning points from the project

This collaborative project is in its fourth year and has illustrated how industry and animal welfare groups can share unpublished data with the aim of reducing animal usage. Animal welfare has also been improved without compromising scientific validity.

The process used by the Steering Group has been one of 'give and take' with neither the welfare nor the industry representatives having everything. It has required trust on both parts and the overarching principle has been the mutual understanding of animal welfare. When confidence in the group was established then data sharing followed as a natural consequence.

In the early stages of the project it was important to



establish a 'quick win' to give the group impetus to proceed with the more difficult areas. Resolving the differences around group size was an ideal topic, which proved its worth.

The project has demonstrated that a group of individuals can have significant impact on the design of preliminary studies which are outside the regulatory framework. As a result of this collaborative study it is recommended that other companies not represented by the Steering Group review their study designs with the aim of adopting the optimised design with fewer animals. A change of mind set is often required to facilitate such a transition.

Within the regulatory arena the ability to modify study designs is limited. A recent presentation to the CPMP Safety Working Party has confirmed that regulators are more concerned with human safety than animal numbers. In fact, there is some concern that animal numbers for dog studies are already too small. The path to regulatory change is therefore long and arduous for this project but is still considered worthwhile in the interest of animal welfare.

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A European pharmaceutical industry initiative to challenge the requirement for conventional acute toxicity studies

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Summary

A working party representing the pharmaceutical industry was formed in 2003 in order to assess the relevance of the data derived from conventional acute toxicity studies in rodents. The aim of the working group is to facilitate co-operation and data sharing on conventional acute toxicity studies, with the objectives of reviewing how acute toxicity data are gathered and used across the pharmaceutical industry, agreeing a harmonised industry approach for the short term focussing on reduction and refinement, and developing a strategy for challenging the guidelines on the requirement for conventional acute toxicity where lethality is a defined endpoint. This paper summarises results from an initial data sharing exercise and illustrates the value of collaborative projects in implementing the 3Rs in toxicology.

Introduction

The pharmaceutical industry recognises the need to continually assess the design and conduct of toxicology studies. Any assessment will include considering and applying the 3Rs principles (Russell & Burch 1959).

A working party representing the pharmaceutical industry was formed in 2003 in order to assess the relevance of the data derived from conventional acute toxicity studies in rodents. The current group represents 11 European pharmaceutical companies and 3 contract research organisations and is facilitated by the UK Medical Research Council's Centre for Best Practice for Animals in Research (CBPAR), now operating as the National Centre for the 3Rs.

Conventional acute toxicity studies in animals are usually performed to support the registration of any pharmaceutical intended for human use. The main objective of these studies is to identify a dose causing major adverse effects (often involving an estimation of the minimum dose causing lethality), usually in rodents, following a single dose up to a limit of 2000 mg/kg, or the maximum technically achievable. In pharmaceutical drug development this is the only study type where lethality is a defined endpoint as documented in regulatory guidelines (European Parliament 2001, Centre for Drug Evaluation and Research 1996, ICH Japan 1999). The information obtained may give an indication of the likely effects of acute overdose in humans but since it often does not include histopathological or toxicokinetic evaluation its clinical usefulness is questionable. The data may also be used to aid dose selection for other studies and provide preliminary target organ toxicity, although within several companies the acute study is no longer the first toxicology study run and this information may be gained from many other study types, including non-GLP (Good Laboratory Practice) sighting studies.

In recent years, progress has been made in reducing and refining conventional acute toxicity studies. Alternatives to the LD_{50} test have been developed and in 2002, the Organisation for Economic Co-operation and Development (OECD) eliminated the oral LD_{50} (lethal dose in 50% of animals) test from its guidelines for the testing of chemicals. The International Conference on Harmonisation of the Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) has also provided the opportunity to refine acute toxicity tests, with the acceptance of data from dose escalation studies (ICH M3 1997). However, national regulatory guidelines currently still specify the requirement for conventional acute toxicity data for pharmaceutical drugs. The requirements in terms of the species, exposure route and observation period varies between geographical regions as shown in Table 1.

For acute toxicity studies the current European Guidelines do not specifically request a non-rodent species where a) lethality would not be an acceptable endpoint and b) dose-escalation studies would be an acceptable alternative to the conventional acute study. Therefore many European companies conduct conventional acute studies in both the rat and mouse. In addition, the European and American guidelines specify a second route (ensuring exposure). It is worth noting that this is the rare study type where a route other than the clinical route is routinely required. Evidence of exposure on other study types is provided by a toxicokinetic assessment.

The aim of the working group is to facilitate co-operation and data sharing on conventional acute toxicity studies, with the objective of:

i) Reviewing how acute toxicity data are gathered and used across the pharmaceutical industry.
ii) Agreeing a harmonised industry approach to conventional acute toxicity studies for the short term with an objective of refining and reducing this study type
iii) Developing a strategy for challenging the guidelines on the requirement for conventional acute toxicity where lethality is a defined endpoint.

These aims have the support of the European Federation of Pharmaceutical Industries and Associations (EFPIA).

Review of conventional acute toxicity studies

An initial data sharing exercise was undertaken by the working group to compare the design of conventional acute toxicity studies and to review how the data generated is used by internal regulatory and clinical colleagues. All of the companies involved in the working group completed a questionnaire on study designs for pharmaceutical drugs. (Note: anti-cancer drugs and imaging compounds were not included in the survey due to specific requirements and/or the life threatening nature of the disease being treated).

Study objectives

The companies were asked to define why they performed acute toxicity studies (Figure 1).

The main objective of conventional acute toxicity studies identified from the questionnaire is to provide information relevant to over-dosage in man. In order to assess the relevance of the data provided from these studies, the group plans to work with the European Poisons Centre comparing the acute toxicity information from animals with data available in man.

The pre-clinical value of the data provided from these studies is seen as low. There has been a recent shift in the pharmaceutical industry to conduct early non -GLP pilot and safety pharmacology studies to assist in the discovery process so the data these studies provide may be used for dose-selection for repeat dose studies. In addition, target organs are usually identified in the repeat dose studies

Finally, three companies felt there was no clinical or pre-clinical value in the data provided by these studies and therefore conducted the studies for regulatory purposes only.

Acute toxicity package

Companies were asked to provide details of their standard acute toxicity package (Figure 2).

The questionnaire revealed that European pharmaceutical companies are providing at least four different acute toxicity packages. One contract research organisation offers a rodent study by the clinical route only. This answer was not included in the evaluation as it was not clear what additional information the Sponsor may provide. The majority of the companies provide a package that is driven by the European guideline, using two rodent species and two routes of administration (the intended human route of administration) and a parenteral route (four rodent studies in total). However, it is clear that several other companies use minimised strategies successfully even within the confines of the current regulatory guidelines. Based upon this output the working party has identified scope for a reduction in the number of rodent studies conducted and recommends that companies adopt a harmonised approach. In doing this, the working party will address issues relating to the timing of the studies, whether a second rodent species is required and the need for an additional parenteral route where this is not the clinical route.

Timing of studies

The companies were asked when they perform acute toxicity studies. All but two replied prior to first administration in human. This timing is driven by the ICH guideline that specifies the requirement for data prior to first dose in man (ICH Japan 1999). Of the remaining two companies one intends only to perform the studies prior to first dose in man in Japan. The other provides preliminary information prior to first dose in man but only performs the definitive studies during Phase 2 clinical trials. Both of these approaches place the acute toxicity studies later in the development programme and this means there is a reduction in the number of compounds requiring definitive acute toxicity studies due to compound attrition during the development process.

The working party has started to evaluate how acute toxicity data is used to assess safety prior to the first dose in man to establish whether the studies need to be routinely conducted prior to clinical trials.

What data are provided?

The companies were asked to define the data obtained from acute toxicity studies (Figure 3).

In general only very limited data, other than maximum non-lethal dose and minimum lethal doses, is provided. The microscopic evaluation of selected tissues is rare and done on a case-by-case basis.

Number of rodents used per project?

The companies were asked how many rats and mice they used per project in acute toxicity tests. (Figures 4a & 4b). The responses indicate there is large inter-company variability in the numbers of rodents used. This was particularly evident in the use of mice, with three companies not using any mice and four companies using 60 to 100 mice per project. The companies using mice were mainly doing this for regulatory purposes. However, the companies not using mice were those already employing minimised approaches to acute toxicity testing successfully. The data on animal numbers shows there is significant potential for refinement of study design and reduction in the number of rodents used per study.

How useful is the data obtained from conventional acute toxicity studies?

In order to get an initial understanding of the expectations of those that use the data from acute toxicity studies, members of the working group consulted internal regulatory and clinical colleagues to determine their views on the utility of data from conventional acute toxicity studies and in particular the value of mortality data in the absence of organ pathology or toxicokinetics.

Responses from regulatory colleagues indicate that the data is primarily required to ensure regulatory compliance and not because it is necessarily useful. Similarly, responses from clinicians indicate that there is limited clinical value in mortality data alone and that information on the clinical effects of acute overdose is more useful especially if the effects can be monitored. Feedback from clinicians also supports the use of alternative approaches, such as maximum tolerated dose as an endpoint rather than maximum non-lethal or minimum lethal dose, provided a histopathological, biochemical and toxicokinetic evaluation is included.

Ideal acute toxicity package

Members of the working groups were asked to propose their ‘ideal’ acute toxicity package within the confines of the

current regulatory guidelines (Table 2). In most instances, the company ideal is less than their current package and would lead to a significant reduction in the number of rodents used.

Collaboration as a tool for influencing change

Collaborative industry working parties such as this allow the pharmaceutical industry to share data with the aim of reducing animal numbers. In order to achieve this effectively an understanding of both toxicology and animal welfare is a requirement. Sharing such information and then being able to demonstrate the limitations of toxicity tests based upon objective data analysis is a powerful tool for influencing change both within the industry itself but also externally providing a mechanism to optimise toxicity testing in partnership with the regulators.

Conclusion

Over half of the companies involved in the working group follow the European guidelines for conventional acute toxicity testing, using two species (rat and mouse) and two routes of administration. However, the survey also indicated that there are a number of minimised approaches to acute toxicity testing being used successfully within the industry that have already helped to reduce rodent numbers used in this study type. The primary reason for carrying out conventional GLP acute toxicity tests is to provide information relevant to overdose in man and to comply with regulatory guidelines. Initial feedback from internal regulatory and clinical colleagues indicates that the value of mortality data is limited. Together, the results of the survey and the feedback suggest there is scope for reviewing how the data is generated and its scientific value. The immediate aim of the group is to reduce and refine conventional acute toxicity studies in rodents, agreeing upon a standard approach to conventional acute toxicity testing which minimises the number of studies conducted, the number of animals used and the use of mortality as an endpoint. However, the final goal is to replace conventional acute toxicity studies in rodents. Consequently, the working group will review whether single dose data has scientific value in predicting overdose in man. To achieve this more information is required on the value of the acute toxicity studies and the group has begun to compare pathology data from single and repeat dose studies and to consult with the European Poison Centres. In addition, the potential of alternative designs will be investigated. Clearly, it is necessary to seek input from the regulatory authorities and external clinicians and this is the next stage for the group.

There is inconsistency in the approach to providing acute toxicity data within the pharmaceutical industry but by sharing data it has been shown there is both the scope and the willingness to agree upon a harmonised approach to reduce the number of studies and refine the design in the short term, and then to challenge the requirement for these studies (replacement) in the longer term.

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EU	USA	Japan
2 species	2 species, including a non-rodent	2 species, including a non-rodent
2 routes, clinical plus one ensuring exposure	2 routes, clinical plus one ensuring exposure	Clinical route
7-14 days observation	14 days observation	14 days observation

Table 1: Summary of conventional acute toxicity guidelines for pharmaceutical drugs

Species	Administration route	Number of Studies
One rodent acute One non-rodent dose escalation study	Clinical and parenteral Clinical	3
One rodent acute One non-rodent dose escalation study	Clinical Clinical	2
One rodent acute	Clinical	1
One rodent dose escalation study Non-rodent dose escalation study	Clinical Clinical	2

Table 2: Ideal toxicity studies

Figure 1: Objectives of Acute Toxicity Studies

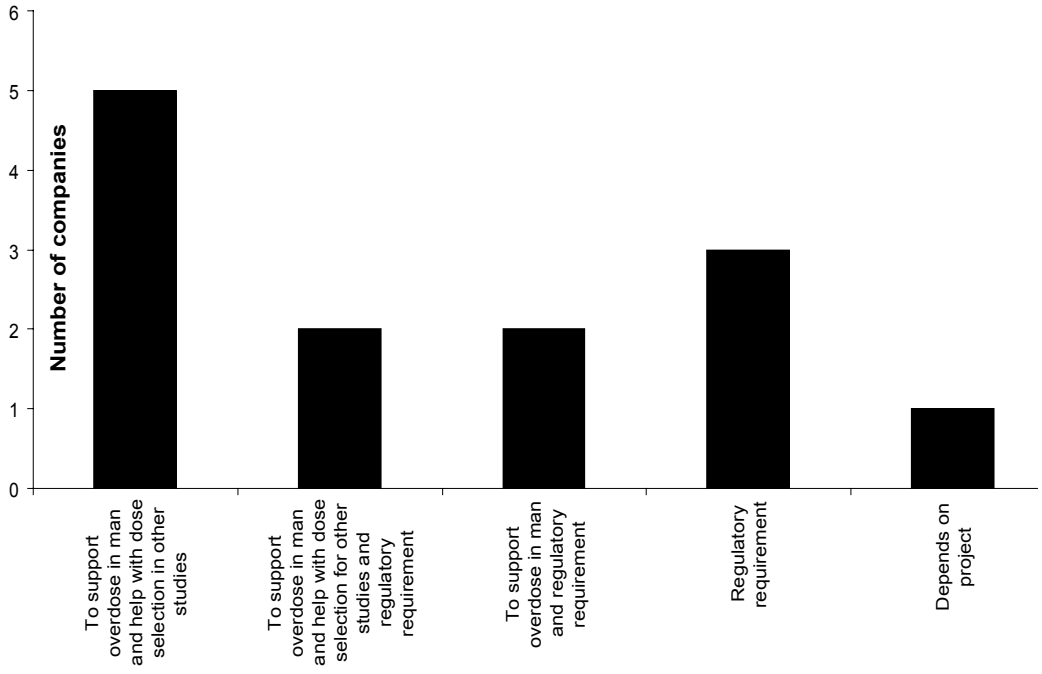


Figure 2: Details of Standard Acute Toxicity Packages

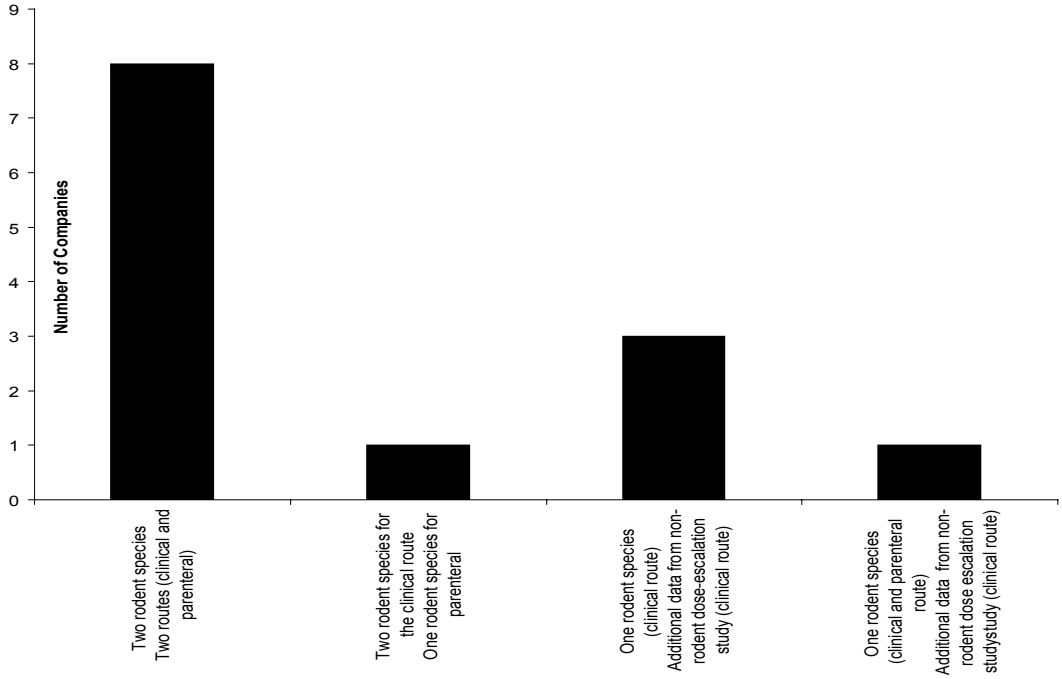


Figure 3: Data obtained from Acute Toxicity studies

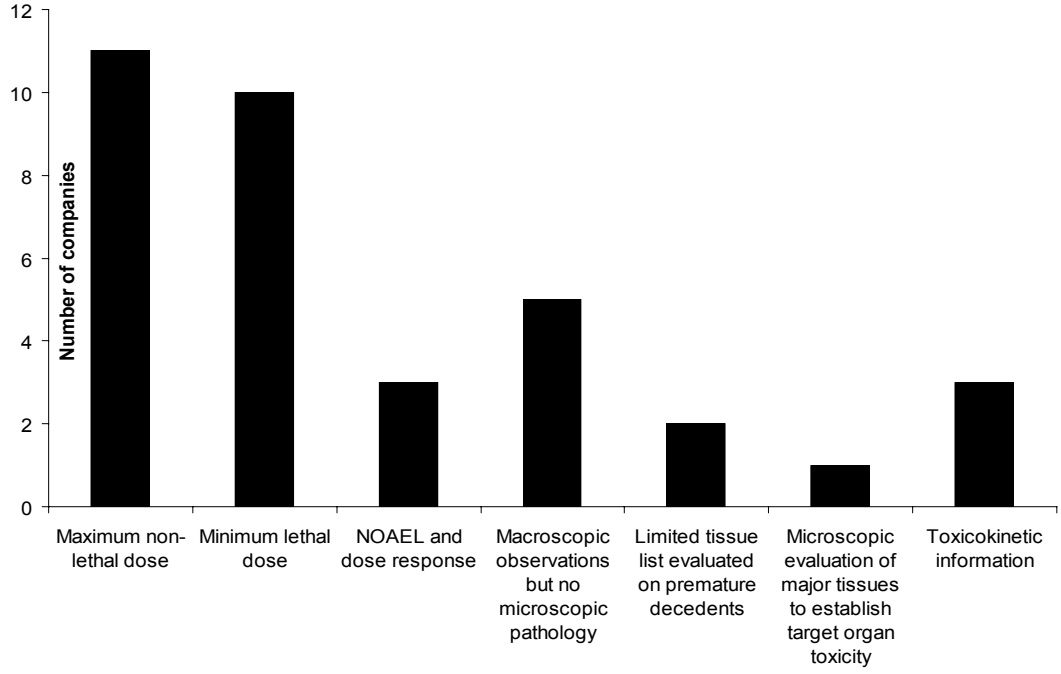


Figure 4a: Rats used per project in acute toxicity tests

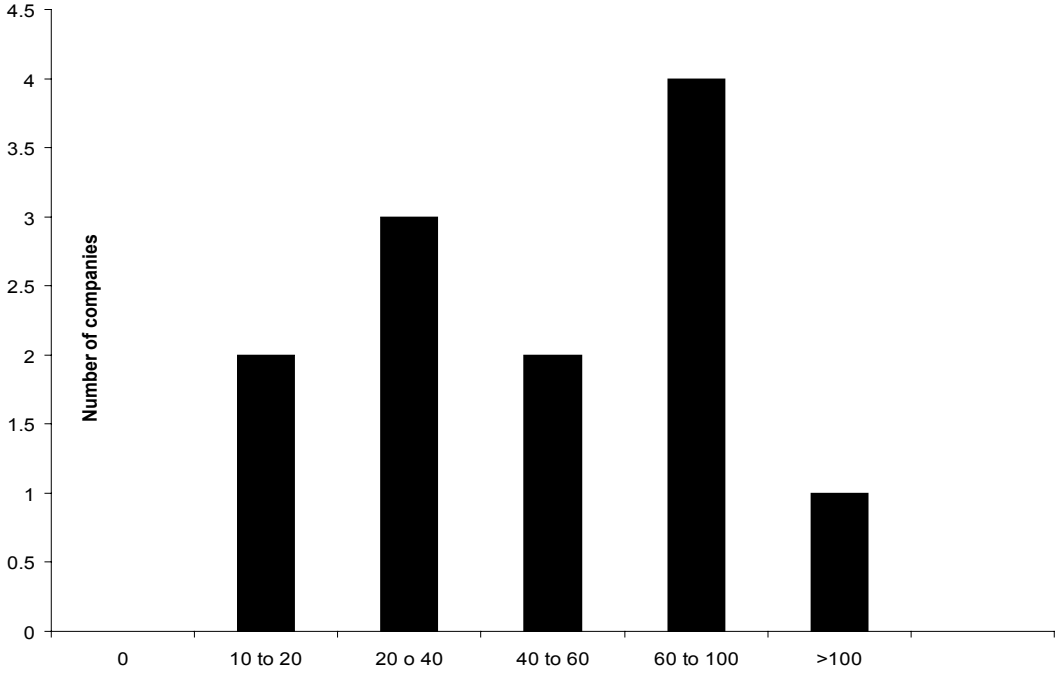
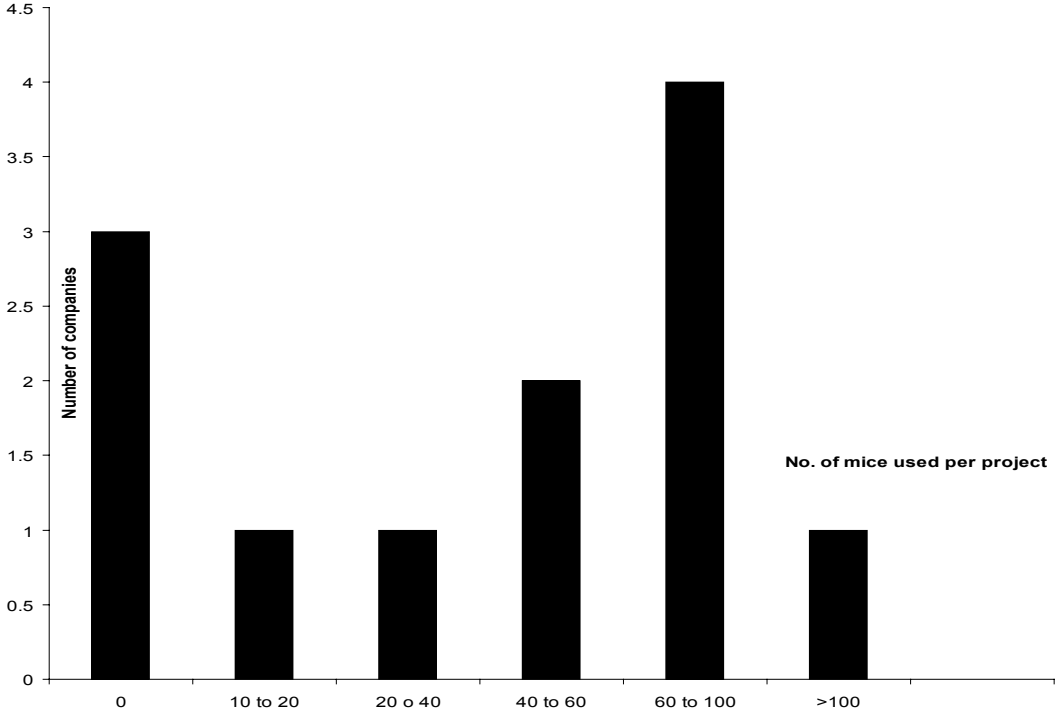


Figure 4b: Mice used per project in acute toxicity tests



Harmonisation of Animal Care and Use oversight across multiple sites in multiple countries

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The 3Rs should be a cornerstone of every animal care and use program. Oversight of animal care and use programs and enhancement of the 3Rs can be facilitated through the use of Ethics Committees. These committees, also called Ethical Review Committees or Institutional Animal Care and Use Committees, are required by national law in many, but not all, countries. However, the OECD Guidelines state: â€œAll aspects of animal studies should be subject to an ethical review process as defined by animal welfare legislation and the ethical oversight groups of the testing organization. Where such legislation is not available, it may be necessary for the laboratory to develop its own ethical guidelines and procedures.â€ The interest in this process is also evident as the topic of several meetings such as ESLAV in Lahti, Finland in 2003 and the June 2002 workshop â€œEthics in Researchâ€ in Pisa, Italy. This presentation will compare and contrast the approaches of several countries in relation to such things as the composition of the committee, functions of the committee and committee procedures. Using performance standards, an approach to developing of a unified structure will be outlined. This structure provides flexibility to assure national compliance yet sets a level of consistency across company facilities to help assure a standardized approach to ethical oversight and awareness of the 3Rs. This standardization also facilitates overall corporate review of activities and encourages the exchange of information and materials.

Quality Systems : Impact on 3Rs?

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Abstract:

Several Quality Systems and accreditation schemes can be applied to laboratory animal related work. The more commonly used are Good Laboratory Practice (GLP), ISO 9000:2000 (ISO) and the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). The systems may differ in some important aspects, but they all coincide on key points that influence replacement, reduction and refinement. This influence is more important if the main focus of the system is the animal itself and it is considered not only as part of the experimental production process, but also as the main customer to be satisfied. Key areas such as the ethical review process, institutional responsibilities, personnel, veterinary care, animal environment, physical plant and even the health and safety program are scrutinized. The improvement in the quality of all these areas has influence in the 3Rs, specially in Refinement and Reduction. As the requirements of these systems are globally very similar, their implementation also serves as a mean of harmonization of laboratory animal care and use.

Key words: quality systems, replacement, reduction, refinement, implementation

Introduction

Quality Systems (QS) have been developed in many production and service industries. They are usually implemented in order to give the customer an assurance of the product or service provided. Laboratory animals are involved in several schemes, either as a means to obtain the product or as a product themselves. These schemes can be divided in three main categories, even though they may converge, and all are subject to any appicable legal requirements:

1. Commercial breeders. The laboratory animal is the means to obtain the product and also the product itself. The main customer is the purchaser of the animal.
2. Commercial laboratories. In this scheme the laboratory animal is a tool used to obtain the final product (drugs, vaccines, etc.). Again, the customer is the buyer of the product. The researcher using the animal can also be considered as an "internal" customer.
3. Academia. Here the laboratory animal is the tool to obtain the final product. In this case, the final product is the experimental data obtained from the animal-related experiments. The main customer of the animal use is the researcher.

Traditionally the implementation of QS in laboratory animal settings has been related to commercial schemes, and aimed to the satisfaction of the buyers of products, including as products the animals themselves. Later, QS have been introduced in academia, with the main aim of satisfying the quality requirements of the researchers. The other reason to implement QS has been the legal requirements of safety studies. In both cases, the laboratory animals have benefited from this implementation as part of the process.

Nevertheless, when the laboratory animals are regarded only as a tool to obtain the final product, their benefit (the impact on 3Rs), is limited. In order to get the biggest impact on 3Rs, the laboratory animal is to be considered as the main customer.

The QS that more generally address the work with laboratory animals are GLP, ISO (AAALAC Int.) (Howard et al, 2004). GLP was created as an assurance scheme for the accuracy of the records and records and results of health, environment and safety studies. ISO is non-governmental, and its aim is to create a quality standard in the accurate recording of procedures, work instuctions and records of business oriented activities, but can be implemented in almost all kind of settings. AAALAC , although not a *de facto* QS, provides a voluntary accreditation program specifically based on laboratory animal care and use.

The philosophy of implementation

Although having different approaches, all these systems impact on the use and care of laboratory animals, and hence the 3Rs. When such animals are used in the production and research process, they participate in a chain of events that generate experimental data. The animal and its genetics, health status, environmental parametres, basic care and experimental manipulations represent the key points of this chain of events, and have the major influence on the other factors of the schemes.

If efforts to increase quality are focused on the animal and the direct processes that affect it, the quality of the experiment is also increased, satisfying the researcher (quality of the data), the buyer of the animal or of the products of the research, and also satisfying the legal requirements, related either to the welfare of the animals or to the GLP.

When the aim of implementation is the certification or an accreditation to obtain legal permits or an enhanced market position, the impact on the 3Rs is limited. These cases are usually business oriented, and the animal unit is a part in a larger scheme. The biggest impact on the 3Rs is possible when the implementation of a QS as a management tool goes beyond minimal standards of laboratory animal care and use. These cases have more to do with voluntary implementation of a QS, and the animal unit is the main or only focus of attention. The implementation of QS generates different levels of planning, bureaucracy and economic expenses. The formula to transform the implementation in real benefit for animals is to apply directly all the required tasks in the daily routine of animal and care use.

As a summary, the impact on the 3Rs will be more important when the animal is considered the main customer and the implementation as a management tool in all aspects of animal care and use, rather than aiming at external customers or regarding the implementation as an obligation.

"Impact" features

How do QS impact on 3Rs in practice? The implementation of a QS can effect all key points of an animal care and use program, including the institutional responsibilities, the ethical review process, the standard operation procedures, the animal environment (primary and secondary enclosures, physical plant), the animal handling, the veterinary care, staff training and qualifications, the equipment and facility maintenance.

Revision and improvement in all these areas can influence the 3Rs, especially in refinement and reduction. With regard to replacement, an approved ethical review process should ensure that no animal experiments are carried out when alternative methods are available. This is also important when animals are used for teaching purposes in academia. Some QS pay special attention to the composition and functioning of these ethical committees. In addition, when inspectors or visitors are specialists in the field, they can suggest replacements or alternative methods.

The influence on refinement is more direct and clear. Any improvement in the animal environment, animal handling, anaesthesia, analgesia, euthanasia, etc. results in refinement of animal care and use. This positive improvement may be a result either of the revision of the animal care and use program when the implentation of the QS is being carried out, or of the corrections required/suggested by inspectors or visitors.

Reduction is obtained mainly as secondary to refinement, but also as a direct effect, through the revision of experimental protocols (e.g. statistical analysis). Refinement in the animal environment, handling, and in many experimental techniques such as anaesthesia results in reduction because of the homogeneity of experimental data and reduction in animal losses, removes the need for repetition of experiments or the use of a greater numbers of animals.

Quality systems and harmonization

Standards and legal requirements for laboratory animal care and use differ between countries, and in some cases within the same country. Legal requirements are applicable only in each political entity, and have no effect in any other. Often people in charge of the implementation of the law are not laboratory animal specialists, and do not have a comprehensive knowledge of the field. This results in a variation in quality level in laboratory animal care and use programs around the world.

However the same QS differ little, can be applied everywhere and are able to be revised by specialists in the field. The level required by QS cannot be lower than the legal requirements, and in some cases exceeds them because QS can take into account aspects that are not specified by law, or because national government inspectors overlook important issues.

When a QS is implemented in places with different standards and different legal requirements, the resulting overall level is higher, or at least very similar. Therefore, it can be deduced that the implementation of QS serves as an effective tool for harmonization and can impact on implentation of the 3Rs in a positive manner.

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Guidelines on Endpoints : a successful case of harmonisation

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Abstract

The Canadian Council on Animal Care (CCAC) has been the national organisation responsible for setting and overseeing the implementation of standards for the care and use of animals in science since 1968. The CCAC pioneered the institutional animal care committee (ACC) as the keystone of its decentralized ethical review and oversight system. Over the past five years, there has been an increasing recognition of CCAC as a quasi-regulatory system nationally and internationally.

Canada was the first country to develop and implement guidelines on endpoints. In 1998, the *CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing* were published in French and in English and elicited the validation by Health Canada of non-lethal endpoints for five biologicals. The endpoints document was recognized as an effective refinement tool along with the *OECD Draft Guidance Document on the Recognition, Assessment and Use of Clinical Signs in Humane Endpoints for Experimental Animals Used in Safety Evaluation* at the 1999 Third World Congress on Alternatives and Use of Animals in Life Sciences. A Spanish edition of the document was published in *Animales de Experimentacion* in 2000.

The guidelines on endpoints emerged as a flexible basis for harmonisation worldwide at the June 2001 International Symposium on Regulatory Testing and Animal Welfare, which was organized by CCAC in collaboration with the International Council for Laboratory Animal Science (ICLAS). This symposium resulted in published proceedings in the *ILAR Journal* in early 2002. The interpretation and implementation of CCAC guidelines on endpoints since 1998 has led to the identification of good practice by scientists, veterinarians, animal care technicians and other stakeholders, some of which were shared with international colleagues at the 2003 Annual Conference of the American Association for Laboratory Animal Science (AALAS).

Using CCAC's guidelines on endpoints as a case study, the peer-based approach underlying the guidelines development process, the use of evidence-based learning loops in the evolution of best practices to implement these guidelines, and the institutional ACCs are described as the three pillars of the international harmonisation of standards for the care and use of animals in science.

Keywords **ethics, endpoints, animal welfare, international harmonisation, refinement**

Introduction

The Canadian Council on Animal Care (CCAC) was established in 1968, following an initiative of the National Research Council (NRC), the Association of Universities and Colleges of Canada (AUCC), the Medical Research Council of Canada (MRC) and the Canadian Federation of Humane Societies (CFHS) to provide public accountability for the use of animals in research, teaching and testing.

Since its establishment, CCAC's activities have been funded principally by public funds through grants from two federal granting agencies, the Medical Research Council of Canada (now known as the Canadian Institutes of Health Research - CIHR) and the Natural Sciences and Engineering Research Council of Canada (NSERC). The CCAC was incorporated as an independent and autonomous organisation in 1982. It functions as a peer review agency, involving stakeholders at all levels of the organisation. This is underlined by the composition of CCAC's Council which now includes 24 national member organisations representing academic and government bodies as well as industry and animal welfare organisations (Canadian Council on Animal Care, 2004).

The purpose of the CCAC is to act on behalf of the people of Canada to ensure, through programs of guidelines development, assessment and education/training/communications, that the use of animals in Canada, where necessary for research, teaching and testing employs physical and psychological care according to acceptable scientific standards, and to promote an increased level of knowledge, awareness and sensitivity to the relevant ethical principles (Canadian Council on Animal Care, 2004). The underlying ethical basis of all CCAC guidelines and policies requires adherence to the Three Rs (Reduction, Replacement and Refinement), first outlined by Russell & Burch (1959). The concept of the Three Rs underlies the standards adopted in a large number of countries, governing the treatment of animals in science.

The CCAC pioneered the system of local institutional animal care committees (ACC) as the keystone of its decentralized ethical review and oversight system. ACCs are now an essential part of oversight systems worldwide, irrespective of the voluntary or legislated frameworks in place in different jurisdictions. Brown (2004) has described the generic structure of ACCs.

CCAC as a quasi-regulatory, standard setting organisation

A legal opinion commissioned by CCAC (Wilson, 1998) concluded that under the *Constitution Act 1867*, the federal government does not have jurisdiction to legislate with respect to experiments involving animals as this is a provincial responsibility in Canada. Whilst CCAC standards had begun to be referenced by some provinces in the regulations to their respective animal welfare legislation prior to 1998, concerted efforts by CCAC and its constituents initiated in 1999 catalyzed this emerging trend, so that five of the six provinces that have legislated in the matter, now make reference to CCAC standards in their regulations or their legislation (Canadian Council on Animal Care, 2004).

The mechanism through which the federal government has lent its support to the humane treatment of animals used for scientific purposes is not strictly speaking legislative in nature. However, in many respects this mechanism represents one of the most powerful instruments available to the federal government for setting national standards. The federal government's power to provide for grants subject to conditions imposed on the recipients, be they provincial governments or individual or corporate recipients, may take a

variety of different forms. One form is that of the conditional federal grant or contract. This manifestation of the federal power is what currently underpins the imposition of CCAC standards on facilities receiving funding from the Canadian Institutes of Health Research and the Natural Sciences and Engineering Research Council. Where the government itself awards a contract on an academic or non-academic institution, clause A9015C of the *Public Works Standard Acquisition Clauses and Conditions Manual* imposes conditions related to the care and use of experimental animals in public works and government services.

The CCAC system of oversight was originally created as a purely voluntary system. Nonetheless, progressively, its guidelines are being turned into standards by the users themselves, including provincial and federal regulatory agencies. The end result is a hybrid system of oversight, robust enough to ensure compliance with its standards, whilst remaining inclusive and flexible by the nature of its guidelines development process. The exact nature of the Canadian system of oversight has been captured as part of a recent review of progress achieved in the implementation of the Three Rs in European countries, Canada and the United States (De Greeve *et al*, 2004).

Guidelines on endpoints: emergence and international recognition

The CCAC *Ethics of Animal Investigation* (Canadian Council on Animal Care, 1989) requires investigators to follow the Three Rs. The investigators responsibility in terms of refinement ("to reduce to an absolute minimum the amount of distress imposed on those animals that are still used") is clearly expressed in this policy statement. Minimizing potential pain and distress and maximizing animal well-being are the ethical drivers for refinement measures.

Categories of invasiveness describing the potential level of pain and distress that could be experienced by animals involved in experimental procedures, were developed by CCAC in 1988, and revised in 1991 (Canadian Council on Animal Care, 1991). In 1996, CCAC began to report numbers of animals used according to five purposes of animal use. In 1997 (see Figure 1) it was found that 29% of animals used in Canada for research, teaching and testing experienced moderate to severe pain and/or distress, categories D and E on CCAC's five-point scale. It is for these types of studies that the CCAC *guidelines on: choosing and appropriate endpoint in experiments using animals for research, teaching and testing* (Canadian Council on Animal Care, 1998) were developed. The guidelines provide a specific definition of an endpoint and give specific guidance establishing earlier endpoints, recognizing the following areas where earlier endpoints are desirable:

- Monoclonal antibody production
- Cancer research
- Acute toxicity testing in mammals
- Acute toxicity testing in fish
- Chronic toxicity studies
- Aging
- Pain research
- Infectious disease studies, vaccine trials, etc.

The purpose of the guidelines is (i) to provide guidance for selecting an endpoint that reduces the potential for animal pain and/or distress, whilst still satisfying the experimental design requirements for objective evaluation, and (ii) to assist institutional ACC members and investigators in fulfilling their ethical responsibilities in minimizing animal pain and/or distress. Key provisions of the guidelines include: recommended procedures for selecting an appropriate endpoint; using preliminary or pilot studies to determine the appropriate endpoint; determining the required frequency of animal observations; defining responsibility for animal observations; and training of personnel in clinical animal observations.

At the national level, the implementation of these guidelines had major qualitative and quantitative impacts as a refinement tool. It elicited the validation of non-lethal endpoints for five biologicals by Health Canada (Calver *et al*, 1999). As evidenced at the time of CCAC assessment visits of institutional animal care and use programs, the implementation of the guidelines has increased the attention paid by animal users to animal well-being and has fostered a team approach involving scientists, veterinarians, animal care technicians and ACC members. On the quantitative front, in 1998, the implementation of the new guidelines resulted in a 50% decrease in the numbers of animals reported to be used under Category of Invasiveness E (see Figure 2).

At the international level, the *CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing* were recognized as effective refinement tool with the OECD *Draft Guidance Document on the Recognition, Assessment and Use of Clinical Signs in Humane Endpoints for Experimental Animals Used in Safety Evaluation* (Organisation for Economic Cooperation and Development, 1999), at the 1999 Third World Congress on Alternatives and Use of Animals in Life Sciences (Griffin and Koeter, 2000). Unlike CCAC guidelines, the OECD guidance document addresses the principles of humane experimentation applicable to animals used in toxicity testing studies only. In 2000, a Spanish edition of the CCAC guidelines was published in *Animales de Experimentacion* (Canadian Council on Animal Care, 2000).

The guidelines on endpoints also emerged as a flexible basis for harmonisation worldwide at the June 2001 First International Symposium on Regulatory Testing and Animal Welfare organized by CCAC in collaboration with ICLAS. This Symposium attracted 160 scientists, regulators and animal welfare representatives from 22 countries and resulted in published proceedings in the Institute for Laboratory Animal Research *ILAR Journal,* including specific recommendations on the implementation of current best scientific practices relating to endpoints as well as requirements for future progress (Combes *et al*, 2002). In terms of implementation of current best practices, the following key recommendations were made at that time:

- Information on humane endpoints should be provided on intranet and internet sites;
- extreme endpoints should be avoided wherever possible;
- useful criteria for endpoints should be standardized; and
- the concept of humane endpoints should be introduced into animal user training programs.

The interpretation and implementation of guidelines on endpoints in Canada and in other countries since 1998 has led to progress in the identification of good practices by scientists, veterinarians, animal care technicians and other stakeholders. Some of these practices were shared with international colleagues at the 2003 Annual Conference of the American Association for Laboratory Animal Science with the objective of stimulating the identification of other good practices. Subsequent peer-review and other scientific exchanges should encourage the evolution of these methods into best practices.

Furthermore, representatives from several international and national scientific organisations participating in the First ICLAS Meeting for Harmonisation of Guidelines held in Nantes, jointly with the Federation of European Laboratory Animal Science Associations (FELASA) meeting, agreed to retain CCAC guidelines on endpoints and the OECD guidance document as potential international reference documents (International Council for Laboratory Animal Science, 2004).

Conclusion

At the same time that the guidelines on endpoints were successfully ending their journey towards international harmonisation, the CCAC Council was adopting its *Five-Year Plan 2004-2009*. A major conclusion reached in the Plan, which is likely to be shared by other organisations responsible for overseeing the use of animals in science, is that international harmonisation of standards is one of the two overriding priorities for the CCAC Guidelines Development Program. International harmonisation of standards is a priority because of:

- broad implications for international scientific collaboration;
- global acceptance of research data; and
- international trade.

As was the case for the CCAC guidelines on endpoints, several vehicles are available to foster the harmonisation of standards at the international level, such as the Organisation for Economic Cooperation and Development, the International Convention on Harmonisation, the Council of Europe, AALAS, the Institute for Laboratory Animal Research, FELASA, and others. However, few offer as much potential to catalyze harmonisation and to act as an effective harmonisation platform as ICLAS did through the 2001 CCAC-ICLAS International Symposium on Regulatory Testing and Animal Welfare, the 2002 AALAS-ICLAS Summit of the Americas, the 2003 ILAR-ICLAS Harmonisation Workshop, and the 2004 FELASA-ICLAS Symposium/Meeting on Harmonisation.

The Central role of the ACC

While an effective harmonisation platform is a key structural element of the harmonisation process at the international level, the keystone of the whole enterprise remains the local, institutional ACC. After having thoroughly reviewed the best scientific practices for animal care committees and animal use oversight, participants in the ICLAS-CCAC International Symposium on Regulatory Testing and Animal Welfare (Richmond *et al*, 2001) concluded:

"Experience has shown that different frameworks [voluntary or legislated] provide effective oversight in different jurisdictions and within organisations with different cultures. Indeed, providing the process works in practice, diversity, which can of itself promote continuous improvement, should not be discouraged... Future progress requires the following: encouraging diversity; networking ACCs to identify, encourage and share best practices."

In conclusion, as was the case for the acceptance of both the CCAC guidelines on endpoints and the OECD Guidance Document by the international community, international harmonisation of standards is needed, not international standardization. In that process, the institutional ACC, also called IACUC or Ethical Review Processes in other countries, has a central role to play because:

- it is representative of the scientific culture and moral values of home countries;
- it facilitates communications and empowers informed decision-making at the local level;
- it is already integrated as an accountable keystone of most national oversight and regulatory systems worldwide; and
- it provides each nation with enhanced ability to influence international harmonisation of best practices for animal care and use in science (Gauthier, 2002).

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Harmonising nutrition guidelines supports standardisation and reduction

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Summary

The National Research Council (NRC) has published valuable guidelines based on available scientific data on the minimum nutrient requirements for various species (available on www.nap.edu under "nutrient requirements"). In natural-ingredient diets these recommended levels are usually exceeded, and one of the reasons for doing so is to prevent the possible risk of nutrient deficiencies after longer storage periods. As the NRC guidelines are based on obtaining maximum growth, which is not necessarily the same as optimal health, exceeding these recommended levels may have a negative impact on health. High levels of certain nutrients may induce pathological lesions, which can lead to an unnecessary increase in the number of animals used. Also, imbalances in the relative amounts of nutrients in chow diets can influence the behavioural development in mice (Wainwright, 2001). Due to variation in natural ingredients, variation in dietary composition of chows from different sources and between batches of diets from the same source arise (Ritskes-Hoitinga & Chwalibog, 2003). This will increase variation in experimental results and increase the numbers of animals used. Upon isolating renal resistance vessels from rabbit kidneys for *in vitro* studies in our laboratory, nephrocalcinosis (NC) occurred in a variable frequency and severity. In early 2000, 40% of rabbit kidneys could not be used for the intended purpose. From the second half of 2000 until the beginning of 2004, kidneys from all euthanased rabbits were routinely examined for NC by histological examination. In 16% of the rabbits the NC was of such a degree that the renal resistance vessels could not be isolated. In 13% a light degree of NC was present, which may interfere with the single nephron passage time, as has been demonstrated in the rat. Two batches of natural ingredient diets were analysed and revealed a dietary P level of 0.6% (Ritskes-Hoitinga *et al*., in print). In a rabbit study, purified diets with 4 levels of P (0.1, 0.2, 0.4 and 0.8%) were fed for an 8wk period during the growth phase. The outcome showed a positive relationship between the amount of dietary P in the diet and the severity of NC. The outcome indicates that the recommended dietary P level of 0.22 % (NRC 1977) should be regarded as a maximum level, instead of a minimum level. A dietary P level of 0.1% virtually prevented NC, without compromising bone mineralisation (Ritskes-Hoitinga, *et al*., 2004). Lowering dietary P level in rabbit diets to the current NRC guideline or lower, gives an expected reduction of at least 16% in the number of rabbits used for these kidney physiological studies.

Introduction

The influence of nutrition in experimental studies is underestimated. In many articles the only reference to diet is that a commercial chow diet from a particular firm with a particular product name is used, without further details on the dietary composition. Within the normal variation of essential fatty acid content found in laboratory (natural ingredient) diets, behavioural development of mice can be influenced (Wainwright 2001). It is therefore important that a detailed dietary characterisation should be part of the methodological description of published studies in order to interpret brain and behavioural development in mice (and particularly GM strains) reliably (Wainwright 2001). A batch analysis certificate mentioning nutrient and contaminant levels, should be provided routinely with each diet delivery, and not only on request for an additional charge, which is currently the case. If all customers shared the costs of batch analyses this would not pose a large extra financial burden upon each individual customer.

Kidney calcification in rabbits on natural ingredient diets

A pathological problem in rabbit kidneys was encountered at our laboratory during the dissection of renal resistance vessels for further *in vitro* physiological studies. About 40% had to be discarded in the first half of the year 2000 due to excessive calcifications. Histological sections revealed the presence of calcified deposits in the cortex and medulla. In the remaining 60%, a variable degree or no calcification was seen. Individual variation may interfere with experimental results and can cause a higher standard deviation, making it necessary to use more animals. In rats it has been shown that the presence of calcium deposits can interfere with kidney function and prolong single-nephron passage time (Al-Modhefer, *et al*. 1986). As dietary P is an important etiological factor in NC in rats (Ritskes-Hoitinga, *et al*. 1989, 1992), dietary P was analysed in the chows used at the breeder and the research facility. Dietary P levels were 0.6% (wt/wt) in both batches analysed (Ritskes-Hoitinga, *et al*. in print). The NRC for rabbits (1977) advises that the minimum recommended dietary P level should be 0.22%. All dietary P is expected to be available to the rabbit, due to the microbial activity in the intestines, in contrast to the situation in rats and humans, where phytate P is not available to the organism. The importance of the dietary P level in the etiology of nephrocalcinosis in rabbits was established in a study using purified diets.

Kidney calcification in rabbits on purified diets varying in dietary P level

In rats a positive relationship between the level of dietary P and the occurrence of NC was demonstrated, which led to an adaptation of the NRC guideline for dietary P from 0.4 to 0.3% (NRC 1995). By using purified diets, it was demonstrated that kidney calcification in young male New Zealand White (NZW) rabbits became more severe and occurred in a higher incidence at increasing dietary P levels

(0.1, 0.2, 0.4 and 0.8 % P, at a constant dietary Calcium level of 0.5%; Table 1; Ritskes-Hoitinga, *et al*., 2004). At a dietary P level of 0.1%, kidney calcification was virtually prevented, whereas bone mineralisation was not negatively influenced. This may imply that the current NRC guideline for dietary P of 0.2% for rabbits should be considered as the maximum recommended level instead of the minimum recommendation and may even need to be lowered to a level as low as 0.1% P. At 0.1% dietary P, some NC could be found in the medullary region of a few animals as there is sediment in the rabbit urine, these deposits may be a natural phenomenon. The detrimental effect of relatively high levels of dietary P is well-known in diseased kidneys: dietary P intake restriction slows down deterioration of renal function in progressive renal insufficiency in animal models (Nagano, *et al*. 2003).

(See Table 1)

Kidney histological results of rabbits on natural-ingredient diets

Table 2 gives the results of the histological analysis of the kidneys of 216 rabbits examined from the second half of 2000 until the beginning of the year 2004 (Ritskes-Hoitinga, *et al*., in print). Cortical NC scores of 2 and 3 were found in 16% of all animals, which made these animals unsuitable for the intended purpose. The 2 chow diets used for feeding these rabbits had a dietary P level of 0.6 %, which is three times higher than the minimum recommended NRC level (1977). The individual variation in the degree of NC may be the result from batch-to-batch variation and/or the interaction of the diet with the genetic background of individual animals. As the NZW strain is outbred, the genetic background of each individual is different. By reducing the dietary P level in natural ingredient diets to a maximum of 0.22%, a reduced number of animals will be needed for these *in vitro* studies. If the same applies as in rats, a low enough dietary P level will virtually prevent NC, regardless of the genetic background (Ritskes-Hoitinga, *et al*. 1992). At the same time, less variable results (of kidney physiological and pathological measurements) are likely to occur. This will also result in a reduced number of animals needed for statistical significance.

(See Table 2)

Conclusions

It is recommended to use the NRC guidelines for obtaining standardised dietary compositions as these are the best documented recommendations available. Moreover, these are revised as new scientific data become available. A more consistent use of NRC guidelines will increase standardisation, reproducibility of studies and harmonisation. As NRC guidelines are based on obtaining maximum growth, which does not automatically imply good health, these guidelines should perhaps be regarded as target or maximum levels, instead of minimum levels. Our results in rabbits indicate that the current NRC recommendation for dietary P of 0.22% should be regarded as a maximum level. The use of purified diets provides a better basis for standardisation than natural ingredient diets. By following the AIN-recommendations (American Institute of Nutrition), a harmonised, purified dietary composition for rodents is achieved (Reeves, *et al*. 1993). A detailed dietary description should become a mandatory requirement for all publications involving animal studies.

Table 1: The occurrence of NC in cortex and medulla of growing male NZW rabbits being fed purified diets with different dietary P levels for an 8wk period (Ritskes-Hoitinga, et al. 2004).

Dietary P level (%)	0.1	0.2	0.4	0.8
NC score cortex	0.0	0.13	0.44	1.75
Incidence	0/8	2/8	3/8	8/8
NC score medulla	0.25	0.50	1.56	1.81
Incidence	3/8	3/8	8/8	8/8
% Ca in kidney	0.05 ± 0.01	0.07 ± 0.0 4	0.34 ± 0.65	1.40 ± 1.51

The score is an average for 8 animals for each dietary group.
Kidney sections were stained by Von Kossa.
Score 0 = no calcification;
Score 1 = a few calcifications in the entire kidney;
Score 2 = a moderate degree of calcification;
Score 3 = a severe degree of calcification.

Table 2: Histological analysis of rabbit kidneys for use in 'in vitro'

Incidence NC Cortex	63/216 = 29%
Mean score Cortex	0.5
Incidence NC Medulla	105/216 = 49%
Mean score Medulla	0.7

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International Harmonisation of Care and Use Issues

An approach toward international harmonisation: the care and use of fish

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Abstract

The Canadian Council on Animal Care (CCAC) develops guidelines on issues of current and emerging concerns in response to: the needs of the scientific community; advances in animal care; and the needs of the CCAC Assessment Program. The CCAC Guidelines Program is also charged with ensuring international harmonisation of its guidelines' documents. Guidelines are developed by subcommittees of experts, and are based on "sound scientific evidence". International harmonisation becomes challenging when there is little scientific certainty, and where interpretation of scientific evidence is different in other jurisdictions. Often these differences arise in areas where recommendations to the community are most needed, to provide assistance to both investigators and animal care committees on how best to balance the well-being of experimental subjects and the goals of scientific research.

The process for drafting the CCAC G*uidelines on*: *the care and use of fish in research, teaching and testing* (in preparation) will be used as an example of the development of guidelines in the face of scientific uncertainty (Sandoe, *et al*, 2004) as an example of the employment of a precautionary approach, in attaining international harmonisation. Fish are now one of the most commonly used laboratory animals in Canada. However, what constitutes well-being for fish is an emerging field with often conflicting scientific data presenting unique challenges in guidelines' development.

Keywords: animal welfare, ethics, fish, pain and distress, precautionary approach, international harmonisation

Introduction

The Canadian Council on Animal Care (CCAC) is the national organization with the responsibility for overseeing the care and use of animals in Canadian science. The CCAC system is an evidenced-based learning loop model comprising of three interrelated programs: the Assessment Program, the Education Training and Communications Program, and the Guidelines Development Program (see Figure 1). CCAC guidelines are developed on issues of current and emerging concerns in response to: the needs of the Canadian scientific community; advances in animal care; and the needs of the CCAC Assessment Program. Two principal audiences are targeted by the guidelines: investigators who require information on the care and maintenance of animal subjects as well as the ethical acceptability of procedures to be carried out; and animal care committees (ACCs), responsible at the local level for reviewing animal use protocols and monitoring animal care and use.

In this respect, CCAC's ethical review system is designed to operate at the local institutional level by integrating the needs of scientists, animals and the community through ACCs (Canadian Council on Animal Care, 2000), and to operate at the national level by setting standards for the care and use of animals in science.

Guidelines development process

CCAC is a peer-based organization involving scientists, veterinarians and other animal care personnel, and community representatives at all levels of its operation. Guidelines are developed by subcommittees of experts, peer-reviewed by additional pools of experts, both nationally and internationally, and subject to a widespread review involving constituents of the CCAC system and any parties likely to be affected by the guidelines (see Figure 2). The CCAC Guidelines Program strives for international harmonisation of guidelines while ensuring that the guidelines meet the requirements of the Canadian context.

CCAC and the 3Rs

The principles of the 3Rs (reduction, replacement and refinement), first outlined by Russell & Burch (1959) have become enshrined in legislation regulating the use of animals for scientific purposes in several countries. In Canada, where there can be no federal legislation in this area due to the Constitutional division of power (Wilson, 1998), the CCAC as the national quasi-regulatory body has incorporated these principles into its fundamental policy document *The Ethics of Animal Investigation* (Canadian Council on Animal Care, 1989). For CCAC, the principles of the 3Rs are stated as:

"The use of animals in research, teaching, and testing is acceptable ONLY if it promises to contribute to the understanding of fundamental biological principles, or to the development of knowledge that can reasonably be expected to benefit humans or animals. Animals should be used only if the researcher's best efforts to find an alternative have failed. A continuing sharing of knowledge, review of the literature, and adherence to the Russell-Burch "3R" tenet of "Replacement, Reduction and Refinement" are also requisites. Those using animals should employ the most humane methods on the smallest number of appropriate animals required to obtain valid information."

The CCAC *Ethics of Animal Investigation* (Canadian Council on Animal Care, 1989) requires that pain and distress

be minimised for any individual animal. In particular, certain procedures are deemed to be unacceptable (e.g. use of muscle relaxants or physical trauma without anaesthesia) and special caution is required for other types of studies (e.g. studies on stress and pain, studies involving food and water restriction). Further limits on harms have been established, and a process for establishing endpoints to minimise pain and distress has been detailed in the CCAC G*uidelines on: choosing an appropriate endpoint in experiments using animals in research, teaching and testing* (Canadian Council on Animal Care, 1998).

In accordance with the principles of the 3Rs, all CCAC guidelines seek to provide recommendations that minimise pain and distress arising as a result of experimental procedures carried out on the animals as well as recommendations that focus on improving animal well-being.

CCAC guidelines – a best practice approach

CCAC guidelines are first and foremost based on sound scientific evidence. In line with policy generated by the Guidelines Committee (one of the five standing committees of CCAC, responsible for overseeing the Guidelines Development Program), every guideline statement should be fully justified, including reference to the published literature as far as possible. In addition, the iterative process of CCAC guidelines' development ensures that recommendations made by expert members of the subcommittee responsible for the development of the guidelines' document are subject to peer review by an additional group comprising both national and international experts in the area, plus a further review by the constituency at large (see Figure 2). For CCAC, peer review also includes community representatives and members of the animal welfare community, mainly through the involvement of representatives from the Canadian Federation of Humane Societies. Through this mechanism, the evolution of a guidelines' document takes into consideration both general societal concerns and the interests of the animals.

Nonetheless, the scientific basis to provide an understanding of the impact of procedures or of housing and husbandry on animal well-being is in itself the focus of an emerging area of research. The contexts for the use of animals, be they in the areas of biomedical, agricultural, or ecological research, shift more rapidly than the associated welfare-orientated research (e.g. the rapid increase in the use of genetically-modified animals, prior to a complete understanding of the potential for phenotype abnormalities (Gauthier & Griffin, 2000), or the increase in the use of fish as a research model prior to a complete understanding of the housing preferences of various species of fish (Griffin & Gauthier, 2004)). This is also a function of the process of science itself, as hypothesis driven, with little emphasis placed on firm conclusions, or on transferring knowledge gained into practical applications (Maxwell, 1984; Sandoe, 2004).

International Harmonisation

While CCAC guidelines are based on sound scientific evidence and expert opinion, subject to peer review, taking into account the relevant ethical considerations, CCAC also bears a responsibility to ensure that its guidelines are harmonised with those of the international community. A recent strategic planning exercise conducted by CCAC identified "international harmonisation of guidelines" as one of the top two priorities for the CCAC Guidelines Program. International harmonisation of guidelines is important for CCAC and in particular for Canadian scientists, having broad implications for international scientific collaboration. Assurance that research data has been obtained under conditions that are similar to those adopted by other nations facilitates publication of research in international publications. It also ensures that research scientists, research funding bodies and regulatory agencies from other nations understand the context within which the animal-based studies have been carried out, leading to wider acceptance of research or testing data.

At the outset, in the development of any CCAC guideline, an "environmental scan" is conducted to determine whether guidelines covering the same subject matter already exist, or are in development by other jurisdictions. If so, these may simply be adopted. For example the AVMA Panel Report on Euthanasia (American Veterinary Medical Association, 1993), was incorporated into the CCAC *Guide to the care and use of experimental animals* (Canadian Council on Animal Care, 1993), and the 2000 Report (American Veterinary Medical Association, 2001) is being examined by a CCAC subcommittee for potential adoption, following adaptation to suit the Canadian context. As part of an environmental scanning exercise, contact is made with national and international organizations and experts who have been or are involved in the development of recommendations to guide the care and use of animals in science. For example, in the development of CCAC G*uidelines on: the care and use of wildlife* (Canadian Council on Animal Care, 2003), contact was made with Canadian federal and provincial wildlife directors, as well as international bodies such as the US Ornithological Council and the American Society of Mammologists, among others, to examine guidelines already in existence, and to develop a list of international experts willing to be involved in the review of CCAC guidelines.

Definition of animal and patterns of animal use

All species of vertebrates are covered by the CCAC Program as well as cephalopods. Figure 3 provides an illustration of the numbers of fish, mice, rats and birds used in research, teaching and testing in Canada. These are the most commonly used animals, representing 87% of animals used in Canada (Gauthier, 2004).

Mice accounted for the vast majority of animals used in Canada until 1991, when fishes became the most commonly used taxon. It should be noted that the number of fishes used remained elevated between 1991 and 1996, before decreasing in 1997. This transient increase in the use of fishes corresponds with the enforcement of the Canadian Environmental Protection Act of 1988 (revised 1999) and the resultant transient need to perform increased regulatory testing. There continues to be substantial numbers of fish used in Canada, to support the aquaculture industry; for ecotoxicity testing, as well as for biomedical research. For these reasons, and because of the relative paucity of guidelines addressing the care and use of fish in science, the CCAC Guidelines Committee identified the development of CCAC G*uidelines on: the care and use of fishes in research, teaching and testing* to be one of its priorities.

CCAC Guidelines on: the care and use of fishes in research, teaching and testing

The document is currently under development and has already undergone two levels of review – one review by experts, and a further widespread review in the summer of 2003. It will undergo a third review, by individuals and organizations that have had considerable input at earlier stages in the development of the guidelines.

A good proportion of the guidelines will focus on practical aspects relating to fish well being such as facilities, water quality, and standards for surgical procedures that are not discussed here. Readers are encouraged to consult the CCAC website to access the final publication, anticipated in December 2004 (http://www.ccac.ca).

As an initial stage in the process of developing the CCAC G*uidelines on the care and use of fish in research, teaching and testing*, a review of guidelines already in existence was conducted and organizations involved in developing similar guidelines were contacted. At the time, the American Fisheries Society had already begun work on revising their document G*uidelines for the use of fishes in field research*, to include the care and use of fish in the laboratory setting. These guidelines have subsequently been published (2004). In addition, Appendix A of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes has been undergoing revision to include species-specific provisions for fish. These initiatives provided the CCAC subcommittee on fish with the opportunity to examine the issues emerging in these other jurisdictions, and to examine related scientific evidence.

Three principal areas emerged as issues which will continue to pose challenges for investigators and animal care personnel long after the guidelines are published: procurement of healthy fish; monitoring indicators of well-being for fish; and pain perception in fish. Of these three issues, only pain perception will be examined here as this poses particular challenges in relation to international harmonisation.

Pain perception

In striving to produce a document that will encourage the ethical consideration of fish as a research animal, the CCAC subcommittee developing the guidelines has given considerable thought to the potential for fish to experience pain and distress. However, the subcommittee struggled with the same difficulties outlined by the Fisheries Society of the British Isles in their briefing paper *Fish Welfare*, to the effect that: "The scientific study of welfare is at an early stage compared to work on other vertebrates and a great deal of what we need to know is yet to be discovered" (Fisheries Society of the British Isles, 2002).

It is generally accepted that mammals experience distress, discomfort and pain, and efforts are increasingly being placed on the recognition of pain and distress in laboratory animals (Hawkins, 2002). There are authors nonetheless that continue to challenge claims that non-human species have the capacity to experience pain. Bermond (1997) for instance, has argued that because conscious awareness depends on extensive development of the frontal lobes, few (if any) mammals besides humans possess adequate cortical substrate for pain experience. The CCAC subcommittee, in discussing how to address the issues relating to pain and distress for fishes, were of the opinion that it is important to know whether or not fishes can experience pain, because that may have an influence on the perception of how these animals should be managed, and indeed could influence recommendations made in the guidelines. This is in line with the approach proposed by Duncan (1996) in defining the welfare of an animal. In Duncan's approach, it is not necessarily the state of health or amount of stress that an animal has that matters to its welfare, but the possession and state of a number of cognitive capacities. Therefore, Chandroo, Duncan & Moccia (2004) have argued that if fish are to be given welfare consideration, they must reasonably demonstrate the cognitive characteristics of sentient beings.

Pain in humans has been defined as an "unpleasant sensory and emotional experience associated with actual or potential tissue damage" (International Association for the Study of Pain, 1979). However, the assessment of an animal's emotional experience is impossible. Therefore Bateson (1992), amongst others, has argued that emotion should not feature in the definition of pain in animals. It is most likely that what an animal 'feels' is nothing like the experience of humans with a more complex brain structure; however, that does not mean that the animal's experience is not unpleasant. Key to the discussions of the CCAC subcommittee was the level of importance that should be given to pain and distress for fish, both in terms of their biology and ethics. Determining when fish are in pain or distress is problematic, but an incomplete understanding of pain, distress and nociception in fish does not mean that the issue can be ignored.

Rose (2002), in a review of the literature, came to the conclusion that fish do not have the capacity to experience pain. He based his conclusions on three points:

1) behavioral responses to noxious stimuli are separate from the psychological experience of pain;
2) awareness of pain in humans depends on functions of specific regions of the cerebral cortex;
3) fishes lack these essential brain regions or any functional equivalent, making it untenable that they can experience pain.

More recently, Chandroo, Duncan & Moccia (2004) and Braithwaite & Huntingford (2004) have undertaken reviews of the literature concerning pain perception and arrived separately at the similar conclusion that fishes, at least teleost fish, are more likely to be sentient than not. Like Rose (2002), these authors focused on neuroanatomical, physiological and behavioral evidence to provide indications of pain perception in fish. In particular, a recent series of studies by Sneddon and colleagues have shown: a) that teleost fish possess the same types of pain processing fibres as higher vertebrates (Sneddon, 2002); b) by electrophysiological recordings, that receptors around the head and mouth region of a teleost fish respond to noxious stimuli (Sneddon, Braithwaite & Gentle, 2003); c) that, in comparison to saline-treated controls, teleost fish injected with noxious stimuli did not return to feeding for a prolonged period, had an increased opercular beat rate and displayed anomalous behaviors (Sneddon, Braithwaite & Gentle, 2003); and d) that a fear response (avoidance of a novel object) was reduced in fish that had experienced a noxious stimuli, but could be reversed by morphine (Sneddon, Braithwaite & Gentle, 2004).

While these studies have provided evidence that some fishes are able to perceive and react to noxious stimuli, in order to address the question of whether this matters to the animal, it is necessary to look at these findings in light of the cognitive capacities of the animal itself. Braithwaite & Huntingford (2004) provide a useful discussion of the literature, including some of Braithwaite's studies, to

determine whether fishes have the capacity for complex, flexible learning and memory (Odling-Smee & Braithwaite, 2003). Evidence of ability to generate a mental scene in which diverse information is integrated for the purposes of directing behavior has been viewed as a prerequisite for primary consciousness (Edelman & Tonini, 2000). According to the review by Chandroo, Duncan & Moccia (2004), a substantial body of scientific evidence now demonstrates that fishes have evolved primary consciousness and conscious cognitive abilities.

International harmonisation of the care and use of fishes

The variations of opinions expressed by authors of the recent reviews of the literature in the area of pain perception in fishes (Rose, 2002, Braithwaite & Huntingford, 2004 and Chandroo, Duncan & Moccia, 2004) posed some difficulties for the CCAC subcommittee in determining how to ensure harmonisation with other guidelines under development. In particular, the approach of the American Fisheries Society *Guidelines for the Use of Fishes in Research* is based on the conclusion of Rose (2002) that "This known dependency of the experience of pain on specific cortical structures and the complete absence of these structures or functional equivalents in fishes is a principal point of evidence indicating that the psychological experience of pain is a neurological impossibility for fishes". By contrast the draft species-specific provisions for fish proposed for the revision of Appendix A of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS 123) make no reference to the capacity of fishes to experience pain, as this is implicit, through article 5 of the convention stating that "The member states of the Council of Europe have decided that it is their aim to protect live animals used for experimental and other scientific purposes to ensure that any possible pain, suffering, distress or lasting harm inflicted upon them, as a consequence of procedures being conducted on them, shall be kept at a minimum".

Based on the scientific evidence briefly outlined above, the CCAC subcommittee decided to adopt the approach that fish exhibit the potential to perceive pain, and therefore issued the following guideline: "Fish have the potential to experience pain and manipulations that provoke stress or avoidance/escape behavior may be causes of distress. Researchers have an obligation to mitigate or minimize potential pain and distress whenever feasible and consistent with good scientific practice."

Although differences have emerged in the approach used by the various jurisdictions there are general principles which can form the basis for international harmonisation efforts. The most important of these general principles is that fish should not be subjected to stress, because of the significant physiological and behavioral consequences for the animal (Barton & Iwama, 1991). Therefore, all three sets of guidance referred to in this paper seek to ensure that stress is minimised. Minimisation of stress is important in order to ensure sound scientific data, as only data derived from healthy animals behaving in "normal" fashions can be considered representative of "normal" biological function (American Fisheries Society, 2004). However, minimisation of stress (or welfare consideration) is also important for the animals themselves. For the CCAC, acting on behalf of the Canadian public, there is a broad understanding that the public accepts the use of animals in research, teaching and testing, provided that pain and/or distress are minimised. Therefore, by adopting a precautionary approach, previously outlined by Griffin & Gauthier (2004), that presumes fishes have the potential to experience pain, the CCAC guidelines provide a basis to encourage respect for animal life (Canadian Council on Animal Care, 1999) among those individuals responsible for the care and use of fishes in Canadian science.

Conclusion

CCAC guidelines are developed in response to current and emerging concerns to meet the needs of the scientific community and the CCAC Assessment Program. While the CCAC Guidelines Development Program is charged with the responsibility of harmonising its guidelines with the international community, it is important that this is well balanced with the realities of the Canadian scientific community and the ethos of Canadian society. In addition, the Guidelines Development Program is charged with ensuring that its guidelines are based on sound scientific evidence. Development of CCAC G*uidelines on: the care and use of fishes in research, teaching and testing* (in preparation) provides an example where sound scientific evidence is lacking, and where there is difference of approach emerging between various jurisdictions providing guidance for those involved in the use of fishes in science.

In line with the principles of the 3Rs, CCAC has adopted an approach throughout all its guidelines to emphasize the importance of minimising the potential for pain and distress for individual animals. In this context, a precautionary approach has been used for the CCAC G*uidelines on: the care and use of fishes in research, teaching and testing*, adopting the premise that fish have the potential to experience pain and distress, and building on this foundation to ensure that fish receive the levels of care, monitoring and treatment accorded to any sentient laboratory animal.

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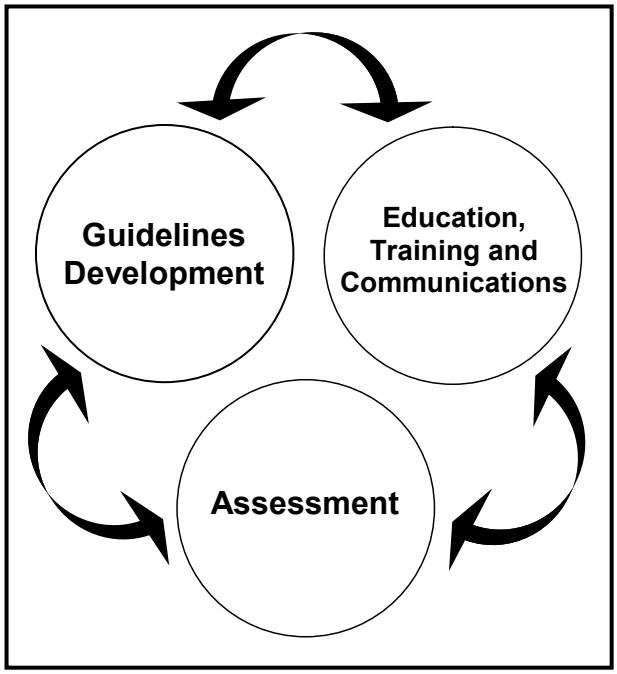
Figure 1: The Three Interrelated Programs of the CCAC System

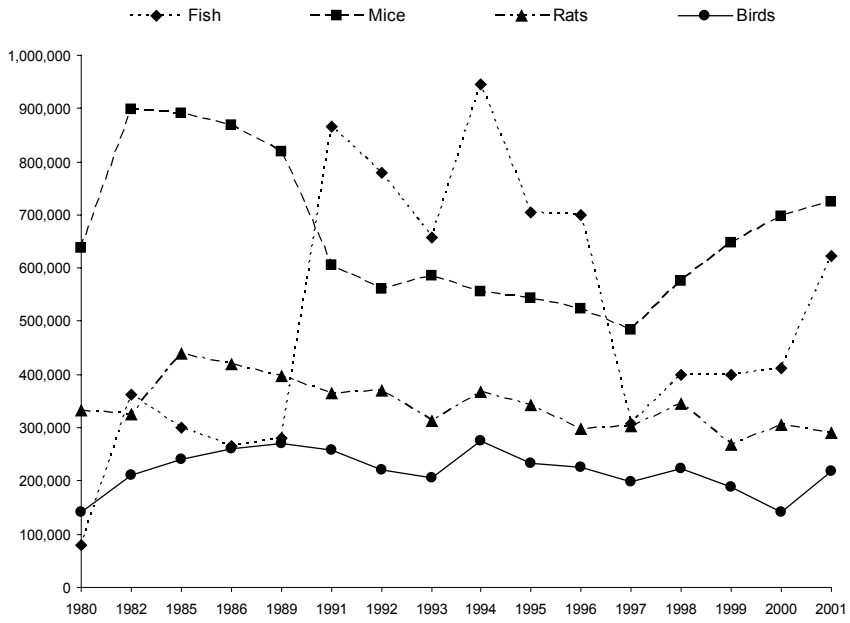


Legend *The CCAC system is comprised of three programs. While each program operates as a stand-alone program, the system relies on feedback from the other programs. For example, feedback from the Assessment Program, identifying difficulties experienced in operating local animal care and use programs at Canadian institutions, helps in prioritizing guidelines development and in targeting educational tools to assist CCAC constituents to meet requirements outlined in CCAC guidelines.*

Figure 2: The CCAC Guidelines Development Process

Legend: *The CCAC Guidelines Development process involves three levels of peer review. A preliminary draft prepared by a CCAC subcommittee, is circulated for review, firstly by known national and international experts; secondly by the first group of reviewers plus CCAC constituents, and other interested parties; and finally by previous reviewers that have contributed significantly to the development of the guidelines. Prior to each review stage, the Guidelines Committee, one of the five standing committees of the CCAC has the responsibility for approving the guidelines document for review, assuring consistency with other CCAC guidelines' documents. Finally the members of the CCAC Council are responsible for approval to publish the guidelines.*

Figure 3: Numbers of fish, mice, rats and birds used in research, teaching and testing in Canada



Basic standards for Laboratory Animal facilities

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Facilities for the care and use of all animals in research, teaching and testing must be conducive to the well-being of the animals, provide an appropriately appointed and safe workplace for personnel and establish a stable research environment. In Canada, the CCAC has recently published guidelines on: laboratory animal facilities â€uidelines provide users and designers of laboratory animal facilities with a tool to assist in achieving optimal levels of animal care, and facilitating good research, without curtailing new and innovative ideas for facility design. Despite the varying needs and many alternative design solutions, there are basic principles that should be considered when designing an animal facility. The flexibility of approach outlined in the CCAC guidelines and described in this presentation could form a useful basis for the international harmonization of laboratory animal facility design

The Council of Europe and the protection of laboratory animals

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The Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS123) is one of the five Conventions of the Council of Europe that are aimed at the protection of animals. Convention ETS123 was adopted on 18 March 1986 and entered into force six months after the 4th ratification, on the 1st January of 1991. The Convention is accompanied by an explanatory report and attached to it are technical Appendices. Appendix A presents guidelines for the accommodation and care of animals. Appendix B contains tables for the presentation of the statistical data on the use of animals for experimental and other scientific purposes.

Convention ETS 123 include provisions concerning the scope, care and accommodation, conduct of experiments, humane killing, authorisation procedures, acquisition of animals, control of breeding or supplying and user establishments, education and training, and statistical information. It is clearly visible from several provisions that the 3Rs of Russell and Burch are used as a basis for the Convention.

Today, sixteen countries have signed and ratified the Convention and thus are Party to the Convention: Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, the former Yugoslav Republic of Macedonia, the Netherlands, Norway, Spain, Sweden, Switzerland, United Kingdom and the European Community. The Convention is signed by Bulgaria, Ireland, Portugal, Slovenia and Turkey.

The Convention provides for Multilateral Consultations of the Parties at least every five years, to examine the application of the Convention and the advisability of revising it or extending any of its provisions according to changes of circumstances and new scientific evidence. The Multilateral Consultations are prepared by a Working Party. For their work, the Parties have invited other Member States of the Council of Europe and non Member States and co-operate very closely with non-governmental organisations representing the fields concerned: scientists, veterinarians, laboratory animal breeders, animal protection associations, specialists in animal science, representatives of the pharmaceutical industry, etc. These non-governmental organisations participate as observers in the meetings. In the preparatory meeting for the 4th Multilateral Consultation the following non-governmental observers participated:

- Canadian Council on Animal Care (*CCAC*)
- European Biomedical Research Association (*EBRA*)
- European Federation of Animal Technologists (*EFAT*)
- European Federation for Primatology (*EFP*)
- European Federation of Pharmaceutical Industries and Associations (*EFPIA*)
- European Science Foundation (*ESF*)
- Federation of European Laboratory Animal Breeders Associations (*FELABA*)
- Federation of European Laboratory Animal Science Associations (*FELASA*)
- Federation of Veterinarians of Europe (*FVE*)
- International Council for Laboratory Animal Science (*ICLAS*)
- Institute for Laboratory Animal Research (*ILAR*)
- International Society for Applied Ethology (*ISAE*)
- World Society for the Protection of Animals (*WSPA*)
- Eurogroup for Animal Welfare (*Eurogroup*)

A representative of the USA participated in the meetings as a non Member State Observer.

Introduction

The participation of representatives of Observer States and non-governmental organisations is of great value. It implies a very broad exchange of information at technical, as well as legal and political levels. Each opinion has to be considered for the elaboration of an acceptable solution intended to improve the level of animal protection. The participation of experts from international professional organizations help Parties to follow technical developments in the different fields covered by the Convent ion and also, to constantly remain attentive to the areas which could possibly influence or be influenced by this field.

Therefore, considering their involvement in this work, they have to be associated with the success of the Multilateral Consultations to ensure common and satisfactory level of protection for animals used for scientific purposes, enabling therefore the Council of Europe to maintain its position of initiator in Europe for the protection of these animals.

Although the working method adopted is time laborious and progress is sometimes difficult, it is the only way to reach consensus on these difficult issues.

Until now, 3 Multilateral Consultations have been held. At the 1st Multilateral Consultation, held in 1992, the Parties adopted a Resolution in which the scope of the Convention was made more precise in respect of genetically modified animals, and certain tables for statistical data were remodelled.

At the 2nd Multilateral Consultation, held in 1993, a resolution on education and training of persons working with laboratory animals was adopted. This resolution contained guidelines for the education and training of persons taking care of animals (Cat. A), persons carrying out procedures (Cat. B), persons responsible for directing or designing procedures and animal science specialists (Cat. D). The guidelines included in the Resolution were based for a great deal on a report that was issued by FELASA.

At the 3rd Multilateral Consultation, that was held in 1997, participants focussed mainly on problems in relation to the transport of laboratory animals including long distance

transport, the acquisition of animals and housing of laboratory animals. Problems related to the import of laboratory animal from States not Party to the Convention and the conditions under which these animals are bred, kept and transported were discussed. Although the Convention does not contain provisions on transport, there are some guidelines on this topic included in Appendix A. Parties agreed that principles on good practice complementing the guidelines given in Appendix A could be elaborated taking into account experience acquired and scientific evidence acquired since 1986. Thus, the Parties adopted a Resolution on the acquisition and transport of laboratory animals.

The revision of appendix A: Developments

The other prominent issue at the 3rd Multilateral Consultation was about the care and accommodation of animals. Appendix A explains and supplements the principles on accommodation and care as adopted in article 5 of the Convention. Unlike the provisions of the Convention itself, the guidelines in Appendix A are not mandatory; they are recommendations. These guidelines are based on knowledge of the seventies/early eighties and good practice. Existing German and US guidelines were used as a basis. The Parties recognized that Appendix A had proven to be of great value and it was widely used as a reference. At the same time however, it was realised that the Appendix had been drafted more than ten years ago. The Parties agreed that new scientific evidence and new experience ever since make it necessary to consider a revision of the Appendix and to define the areas where further research is needed. It therefore agreed, pending this revision, to draft a resolution presenting guidelines for the improvement of the accommodation and care of laboratory animals which would complement the guidelines in Appendix A. The guidelines in the resolution were mainly based on the conclusions and recommendations of the International Expert Workshop on laboratory animal welfare that was held in 1993 in Berlin. It was concluded that the most important area appeared to be the enrichment of the environment of the individual species according to their needs for:

- social interaction;
- activity-related use;
- appropriate stimuli and materials.

Group or pair housing was considered to be preferable to individual housing for all gregarious species, as long as the groups are stable and harmonious. Cages should be structured to enable an activity – related use of the space available and to provide for appropriate stimuli and materials. It was recognised, that guidelines could never replace close and regular observations of the animals involved to make sure that the enrichment initiatives do not have adverse effects for groups or individuals. In addition to general recommendations the resolution included some species-specific recommendation for rodents, rabbits, cats, dogs, (mini-) pigs, poultry and non-human primates. It was agreed that although knowledge was lacking on certain areas, additional general rules on the housing could be elaborated. The resolution on the accommodation and care of laboratory animals was unanimously adopted.

Taking into account the evolution of scientific knowledge and changing circumstances, Parties realised that the technical Appendices might need to be adapted more frequently than its main provisions. However, because these Appendices are an integral part of the Convention, such adaptations could result in complicated amendment procedures. Therefore, a Protocol of Amendment (ETS 170) providing for a simplified procedure for the amendment of the technical appendices to the Convention was drafted and opened for signature in June 1998. Thus, Parties are able to amend the technical Appendices, without formal adoption by the Committee of Ministers.

The revision of appendix A: The process

At the 3rd Multilateral Consultation it was agreed that the revision of Appendix A should be on the agenda of the 4th Multilateral Consultation. To prepare this revision, 4 Expert Groups on rodents and rabbits, dogs, cats and ferrets, non-human primates and mini-pigs were set up, each of them being composed of experts designated by the non-governmental organisations participating, as Observer, in the Multilateral Consultation. Later on, the Expert Group on mini-pigs was extended to an Expert Group on farm animals including mini-pigs and also Expert Groups on birds, fish and amphibians and reptiles were set up. A co-ordinator was designated within each group. In addition, a general co-ordinator was appointed. This general-co-ordinator works closely together with the secretariat of the Council of Europe. The Expert Groups were responsible for their own working method.

The tasks of these Expert Groups were:

- listing for the species concerned, the main questions to be answered with a view to revising Appendix A;
- examining results already available and practical experience acquired which could be possibly answer these questions;
- identifying areas where further research would be needed and setting up a priority list;
- making proposals for amendments to Appendix A, providing information in particular on the ethological and physiological needs of the animals.

These proposals should be based on scientific evidence and/or current good practice. The Expert Groups should also take into account where appropriate the guidelines of the Resolution on accommodation and care of laboratory animals adopted by the 3rd Multilateral Consultation of May 1997.

In general, the proposals were drafted using a stepwise approach:

- first, the physiological and ethological needs were determined and worked out;
- secondly, the minimum spatial and social enrichment that is considered necessary to fulfil these physiological and ethological needs was worked out;
- thirdly, minimum enclosure sizes and space allowances were determined which allow proper spatial and social enrichment.

It was realised, that limits have always to be set arbitrarily, and although they may be justified by science-based arguments, their exact values cannot be scientifically proved. Under most circumstances such values can be thought of as good practice, but may not necessarily be the best practice. Knowledge gained by further research may necessitate changes in the future. The Working Party explicitly agreed that accepted good practice could be used as a basis for suggested modifications.

Where possible and practical, performance based standards have been sought, to encourage and facilitate diversity and innovation.

It is accepted that there must be a careful choice of enrichment methods so that they are compatible with the type of study or use of the animals, and with standardisation of these within a study can help minimise any variation of other interference with results. Care should also be taken to ensure that these will not cause any harm to these animals. Enrichment programmes should be focussed on high priority behaviour that is strongly motivated, such as foraging and social behaviour.

The Expert Groups elaborated a Part A and a Part B document. Part A contains the proposals for the revision of the Appendix. These proposals are based on scientific evidence and accepted good practice and are to be considered as expert recommendations. Almost all the Expert Groups managed to present proposals that were based on consensus, often as the result of extensive discussions within the Expert Groups. This Part A is discussed and subsequently adapted by the Working Party. Once there is a full agreement on the text the discussion is finalised and the final version will be presented to the Multilateral Consultation for approval. Discussion on these final drafts will only be opened if there is new scientific evidence.

Part B documents contain background information based on scientific evidence and practical experiences with the aim to explane and clarify the provisions included in Part A. This background information is provided under the sole responsibility of the expert groups and is separately available. It was agreed that in certain cases, for instance for the documents concerning birds, amphibians and reptiles and fish, given the huge variety of species used for experimental purposes, Part B might also include additional information on species not covered by the species-specific provisions. The purpose of this was to strike a balance between the needs of clarity and readability of Part A and the amount of detailed information available for certain species, by recognising the value and the importance of the information by the various Expert Groups. In order to state more clearly this principle, and to ensure that Appendix A would cover in one way or another also less commonly used species, the Working Party agreed to amend slightly the Introduction to the general Section of Appenix A, and more precisely Paragraph 4, and to include a provision "Further advice on specific requirements for other species (or if behavioural or breeding problems occur) should be sought from experts specialised on the species concerned and care staff, to ensure that any particular species needs are adequately addressed".

A Drafting Group was entrusted with the task to safeguard linguistic consistency of all the guidelines that are to be included in the revised Appendix A.

The finalised documents will be formally adopted at the 4th Multilateral Consultation. The text of the Convention and the related documents, such as resolutions adopted by the Committee of Ministers, as well as the draft proposals for the revision of Appendix on which the discussion is finalised and the finalised of the background documents are available on the website of the Council of Europe.

The revised appendix A: What is in it?

The revised Appendix A will include a General Section providing guidelines on accommodation, housing and care relevant to all animals used for experimental and other scientific purposes. Supplementary guidance concerning commonly used species will be presented in species specific sections along the lines of a standard format. Where no information is included in these specific sections, the provisions of the General Section apply.

There are several differences between the actual Appendix A and the revised Appendix A. Some of them are mentioned below.

- All figures that included in the actual Appendix are deleted, and tables are provided instead;
- The guidelines included in the actual Appendix A were based mainly on biometrical principles. There is not much differentiation between categories of animals of the same species. Minimum enclosure sizes and space allowances are not only based anymore on simple correlations between weight and space, which neglect the different needs of animals of the same species depending on strain, age, sex, reproductive status, etc. Such correlations are not justified by current knowledge. A range of factors affecting the welfare of experimental animals cannot be reduced to purely mandatory regulations and minimum requirements of space dimensions and stocking densities.
- More attention is paid to special cases, categories of animals, sec, age, etc.
- More emphasis is put on social housing. Single housing of gregarious species should only occur if there is justification on veterinary, welfare or experimental grounds.
- More species are covered: in addition guidelines for the housing and care of rodents, rabbits, dogs, cats, farm animals, chickens, quails and non-human primates in general also guidelines on amphibians, reptiles, fish, mini-pigs, other species of birds and non-human primates are included.
- A broader set of guidelines is given for each species: in addition to minimum enclosure sizes and space allowances and enclosure temperatures species specific guidelines are given on issues such as feeding, enrichment, handling, flooring, substrate and bedding, health, cleaning, humane killing and identification.

The Working Party agreed that the guidelines that are included in the Appendix are to be considered as minimum guidelines.

In accordance to article 4 of the Convention, Parties are free to adopt stricter measures than those provided. Although article 5 of the Convention states that "regard should be paid to the guidelines for accommodation and care of animals set out in Appendix A", the implementation of the provisions of Appendix A cannot be interpreted as being mandatory.

The revision of appendix A: Where are we now?

The first Expert Groups started in January 1998. At that time, it was planned to have the 4th Multilateral Consultation in 2000. This appeared to be too optimistic. It appeared however that both for procedural reasons and because the discussions in the expert groups as well as at the Working Parties took more time than expected. At its 4th meeting (8-11 January 2002) the Working Party agreed that discussions on the following documents were finalised:

- General Section;
- Species specific provisions for dogs;
- Species specific provisions for cats;
- Species specific provisions for rodents and rabbits.

At its 5th meeting (8-11 October 2002) the Working Party agreed that discussions on the following documents were

finalised:
- Species specific provisions for ferrets.

At its 7th meeting (9-11 December 2003) the Working Party agreed that discussions on the following documents were finalised:
- Species specific provisions for non-human primates;
- Species specific provisions for birds;
- Species specific provisions for amphibians.

As far as the substantial issues are concerned, the other documents are nearly finalised. The documents on farm animals and reptiles only need a final linguistic revision by the Drafting Group and then the result will be submitted to the next Working Party for final approval. The document on fish will need some further discussion but hopefully the discussions on this document can be finalised at the next Working Party.

Information concerning Convention ETS123 and related issues can be found at: www.coe.int/animalwelfare. There have been long discussions on the question whether or not draft documents should be published already on the website. Some Parties argued that misunderstandings could arise with regard to the legal nature of the documents and that the detailed meeting report, including reference to the positions of each Representative and Observer should also remain confidential. It should be mentioned here however, that in 2001 the Committee of Ministers adopted a policy on access to documents that "transparency should be the rule and confidentiality the exception". At the end of these discussions Parties agreed that drafts finalised by the Working Party should be published on the internet. At this moment the following documents can be found at the website.
- Text of the Convention and of the Protocol of Amendment and in addition to the chart of signatures and ratifications.
- Text of the Resolutions, Recommendations and Declarations that were adopted at the Multilateral Consultations.
- For the last meetings: the Agenda, the Executive Summary of Proceedings, and the List of participants.
- Draft proposals for the General Section and for the species-specific provisions for dogs, cats, ferrets, rodents and rabbits, non-human primates and amphibians. It is mentioned explicitly that these draft guidelines are not currently entered into force and will be adopted by the Multilateral Consultation of the Parties.
- Background information on the draft proposals.
- Statistics available per country.
- Links to non-governmental resources.

Hopefully, it will be possible to have the 4th Multilateral Consultation in the end of 2004 or the early beginning of 2005. However, at least conditions must be fulfilled then. All the Parties must have signed and ratified the Protocol of Amendment then and within the European Community, the Member States must have reached a common position for the negotiation.

First ICLAS Meeting for the Harmonization of Guidelines on the Use of Animals in Science (Meeting for Harmonization of Guidelines)

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Summary

Participants from around the world were invited by ICLAS to meet in Nantes, France on June 13-14, 2004 to discuss the harmonization of existing national and international guidelines for the use of animals in science. The ICLAS Working Group on Harmonization of Guidelines and subcommittees on guidelines, euthanasia and endpoints were created to pursue the work agreed during this first meeting. The ICLAS Governing Board will be disseminating the results of this work from November 2004 onwards.

Keywords: animal use in science, international harmonization, guidelines

Introduction

The international harmonization of guidelines for the use of animals in research, teaching and testing is an emerging issue in the context of the globalization of research. ICLAS, as an international umbrella organization, is well situated to act as a facilitator in this area. Accordingly, ICLAS was pleased to invite a number of representatives from both international and national scientific organizations, which produce or use guidelines for the use of animals in research, to attend the First ICLAS Meeting for Harmonization of Guidelines held on June 13 and 14, 2004 in Nantes, France.

ICLAS is an international non-governmental and non-profit scientific organization, which exists to promote high standards of animal care and use in education, research, testing and diagnosis, to promote good science and foster humane practices in scientific research. It was created in 1956 through an initiative of the United Nations Educational, Scientific and Cultural Organizations (UNESCO), the Council for International Organizations of Medical Sciences (CIOMS) and the International Union of Biological Sciences (IUBS). ICLAS has collaborated with the World Health Organization since 1961. Its composition includes 30 national members, 37 scientific/union members, 34 associate members and 9 honorary members.

In accord with its mission and aims, ICLAS strives to act as a worldwide resource for laboratory animal science knowledge, to be the acknowledged advocate for the advancement of laboratory animal science in developing countries and regions, and to serve as a premier source of laboratory animal science guidelines and standards, and as a general laboratory animal welfare information center.

ICLAS supports the harmonization of animal care and use policies, guidelines and other forms of regulation on a worldwide basis, as a reflection of the globalization of research. This does not mean standardization. ICLAS considers that each country should be able to maintain an animal welfare oversight system that reflects its cultures, traditions, religions, laws and regulations.

Objectives of the First ICLAS Meeting for the Harmonization of Guidelines:

The meeting presented an opportunity to initiate a dialogue on harmonization of a number of published guidelines, with a view to reaching a consensus on the recognition of these guidelines at an international level.

The meeting also presented an opportunity to build regularly scheduled meetings to work on the international harmonization of guidelines.

List of Participants:

- Institute for Laboratory Animal Research (ILAR)
- Canadian Council on Animal Care (CCAC)
- Council of Europe
- Federation of European Laboratory Animal Science Associations (FELASA)
- Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International
- Humane Society of the United States (HSUS)
- Laboratory Animals Ltd.
- International Union of Pharmacology (IUPHAR)
- Sociedad Española para las Ciencias del Animal de Laboratorio (SECAL)
- Association Française des Sciences et Techniques de l'Animal de Laboratoire (AFSTAL).
- Japan
- Argentina
- Italy
- The Netherlands
- Belgium
- Centre Hospitalier Universitaire de l'Université Laval (CHUL)
- Cornell University
- Charles River (USA)
- GlaxoSmithKline (UK)
- Johnson & Johnson (Belgium)
- Other organizations, including the Organisation for Economic Co-operation and Development (OECD), the Universities Federation for Animal Welfare (UFAW), the European Centre for the Validation of Alternative Methods (ECVAM), and the Home Office (UK) signified their support of this initiative, even if they were unable to send a representative to the Nantes meeting.

Agenda of the meeting:

- Opening Session to discuss the importance of harmonization of Guidelines
- Session on euthanasia Guidelines
- Session on endpoint Guidelines

Presentations given during the Opening Session:

- for organizations producing Guidelines: Dr Gilly Griffin (CCAC)
- for organizations using Guidelines: Dr John Miller (AAALAC International)
- for international scientific unions: Dr J.R. Haywood (IUPHAR)
- for the private sector: Dr Guy De Vroey (Johnson & Johnson)
- for developing countries: Dr Cecilia Carbone (Argentina)
- for Japan: Dr Naoko Kagiyama (JAPAN)

Sessions on Guidelines:

During the meeting, two sets of guidelines were discussed to evaluate their possible use at the international level. An ICLAS Working Group on Harmonization of Guidelines composed of representatives of key organizations producing and/or using Guidelines for the use of animals in research was established.

In addition, two subcommittees were formed to examine general principles in relation to the guidelines on euthanasia and endpoints.

Session 1 on Euthanasia

The following Guidelines on Euthanasia were examined as potential International Reference Documents:

- *2000 Report of the AVMA Panel on Euthanasia*, published by the American Veterinary Medical Association.
- *Recommendations for euthanasia of experimental animals: Parts 1 and 2*. This report of the Working Party was prepared for DGXI of the European Commission to be used with Directive 86/609/EEC of 24 November 1986, *on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes* (No L 358, ISSN 0378-6978), February 1996.

The Subcommittee on euthanasia will prepare a document to:

- Outline general principles for euthanasia
- Support key documents as international references
- Provide a table of comparisons to highlight where professional judgment and particular attention by ethics committees is required
- Identify areas where insufficient scientific evidence exists.

The Composition of the Subcommittee on euthanasia is as follows:

- Dr Guy De Vroey, Chair and ICLAS Governing Board member
- Dr Marilyn Brown, Charles River, USA
- Dr Gilly Griffin, CCAC
- Dr Vera Baumans, The Netherlands
- Dr Ronald Charbonneau, CHUL

Time lines for the Subcommittee on euthanasia: The work of the subcommittee should be completed by October 2004 for consideration by the participants in the June 13-14, 2004 meeting. The final document, including the points listed above, will then be presented for adoption by the ICLAS Governing Board in November 2004 in Buenos Aires, Argentina.

Session 2 on Endpoints

The following Guidelines on Endpoints were discussed as potential International Reference Documents:

- *Guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing*, Canadian Council on Animal Care, 1998.
- *Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation*, OECD, 2000.

The Subcommittee on endpoints will prepare a document to:

- Outline general principles for establishing endpoints
- Support key documents as international reference guidelines
- Provide additional references to give guidance in the implementation of general principles in specific areas of research and testing.

Composition of the Subcommittee on endpoints:

- Dr J.R. Haywood, Chair and ICLAS Governing Board member
- Dr Kathryn Bayne, AAALAC International
- Dr Gilly Griffin, CCAC
- Dr Harry Blom, FELASA

Time lines for the Subcommittee on endpoints: The work of the subcommittee should be completed by October 2004 for consideration by the participants in the June 13-14, 2004 meeting. The final document, including the points listed above, will then be presented for adoption by the ICLAS Governing Board in November 2004 in Buenos Aires, Argentina.

Conclusions

The ICLAS Working Group on Harmonization of Guidelines will be meeting every 18 months to pursue its mandate. The next two meetings will be held in conjunction with:

- The American Association for Laboratory Animal Science (AALAS) Meeting in St. Louis, November 6-10, 2005
- The FELASA Meeting in Italy, June 2007.

Following the ratification (November 2004) by the ICLAS Governing Board of the final documents produced by the ICLAS Working Group on Harmonization of Guidelines, a press release describing the decisions of the ICLAS Working Group will be sent worldwide. To ensure that the information will be communicated rapidly and effectively worldwide, a communications subcommittee was created. The members of this subcommittee are: Dr Gilles Demers (ICLAS), Dr Joanne Zurlo (ILAR), Dr John Miller (AAALAC), Dr Cecilia Carbone (Argentina and ICLAS Treasurer), and Dr Jim Gourdon (ICLAS web master).

Report on the ILAR International Workshop on the development of science-based guidelines for laboratory animal care

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Summary

The Institute for Laboratory Animal Research (ILAR) of the National Academies (USA) hosted a meeting in November 2003 in Washington DC titled "International Workshop on Development of Science-based Guidelines for Laboratory Animal Care." The purpose of the workshop was to bring together experts from around the world to assess the available scientific knowledge that can affect the current and pending guidelines for laboratory animal care. Platform presentations focused on a variety of issues, from information exchange on mechanisms for the development of regulations across different countries and cultures to data based scientific studies on the effects of environmental enrichment on research outcomes. In the discussion sessions, participants were tasked with addressing the current scientific literature on the specific session topics, identifying gaps in the current knowledge in order to encourage future research endeavors, and assessing the effects of current and proposed regulations on facilities, research, and animal welfare. Participants had ample opportunities to share research outcomes and viewpoints in the multiple breakout sessions. Summaries of all breakout sessions were presented in the general session. On the final day of the workshop, a point/counterpoint session was held during which a diverse group of speakers presented their cases for and against harmonization of standards. Although some of the speakers had serious reservations about harmonization, most of the panel members expressed positive attitudes about some form of harmonization. A positive outcome of the workshop was the opportunity for scientists and veterinarians from many countries to begin a dialogue with a goal of understanding the basis for the differences in regulatory approaches in laboratory animal care and the hope of continuing discussions on ways to work together toward some type of harmonization.

Key words: international workshop, science-based guidelines, harmonization

Rationale and goals for the Workshop

In November 2003, ILAR held a workshop in Washington DC to discuss the status of laboratory animal care guidelines in the US and other countries. ILAR was established in 1952 as part of the National Research Council to develop and disseminate information and guidelines for the care and use of laboratory animals. ILAR's mission is to develop guidelines and disseminate information on the scientific, technological, and ethical use of animals and related biological resources in research, testing, and education. ILAR promotes high quality, humane care of animals and the appropriate use of animals and alternatives. ILAR functions within the mission of the National Academies as an advisor to the federal government, the biomedical research community, and the public.

The concept for this workshop arose from the International Committee of ILAR Council, a group of experts that advises ILAR about its activities and future projects. Sensing a need to look at the process of regulating animal research in different countries, the International Committee proposed holding a workshop to examine current changes occurring in Europe with the revision of Appendix A of ETS 123 (Convention on vertebrate animals used for experimental and other scientific purposes) (COE 1986), and how these changes might impact regulations in the US. Since ILAR is the board through which the National Research Council publishes the *Guide for the Care and Use of Laboratory Animals* (the Guide) (NRC 1996), and since the Guide is used as the basis for Public Health Service policy on the humane care and use of laboratory animals, ILAR Council deemed it appropriate to examine the issue of revising the Guide in the context of international activities.

The Guide is intended to assist investigators in fulfilling their obligation to plan and conduct animal experiments in accord with the highest scientific, humane, and ethical principles. It has been translated into at least a dozen languages and is used throughout the world as the basis for accreditation by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Recommendations in the Guide, which was last revised in 1996, are based on published data whenever possible; scientific principles, expert opinion and experience with proven methods and practices have been relied upon in the absence of published data. Ideally, in accordance with the principles of the National Academies, all recommendations in the Guide should be based upon scientific evidence; however, in many cases, there are no published data on basic laboratory animal care.

Therefore, given the current status of regulations in the US and Europe, the Program Committee (Table 1) identified the following goals for the international workshop:

- To compare differences in the process for regulation development among countries.
- To examine specific conditions of laboratory animal care and identify gaps in current knowledge in order to encourage future research endeavors.
- To answer the question – Should we harmonize guidelines/standards?

International differences in regulation or oversight of laboratory animals

To address the first goal, individuals from different organizations were invited to identify the issues on an international level. John Miller presented on behalf of AAALAC International, Wim de Leeuw, on behalf of the Council of Europe, and Gilles Demers on behalf of the International Council for Laboratory Animal Science (ICLAS). In the second part of this session, representatives from agencies in different countries or groups of countries

reviewed their regulatory requirements – Nelson Garnett for the Office of Laboratory Animal Welfare at the US National Institutes of Health, Chester Gipson for the Animal and Plant Health Inspection Service at the US Department of Agriculture, Jonathan Richmond for Europe in general, Naoko Kagiyama for the Central Institute for Experimental Animals in Japan, Clement Gauthier for the Canadian Council on Animal Care, and Paul Gilman for the US Environmental Protection Agency. Some points to summarize this session include:

- Regulatory processes are highly variable based on culture and public influence.
- Processes can range from multiple oversight and/or regulations to self-regulation.
- The presentations encouraged better understanding of differences among participants.

Identification of knowledge gaps

The major portion of the workshop was dedicated to examining specific conditions of laboratory animal care and identifying gaps in current knowledge. Each of the next four sessions addressed various aspects of husbandry for laboratory animals. The species included in the discussions were those most commonly used in laboratories – rats, mice, rabbits, dogs, cats and nonhuman primates. Topics included methods for evaluating housing needs and development of standards; environmental controls, (e.g. lighting, noise, ventilation) and their effects on animal homeostasis; and, environmental enrichment. After three of the sessions, participants broke out into smaller groups for more focused discussions. Session leaders presented questions to their groups to center the discussion on identifying gaps in the scientific literature to support the development of guidelines or regulations. Rapporteurs recorded the discussions and presented summaries to the whole group after each breakout session.

While the entire workshop cannot be summarized here, there were some major outcomes related to the goal of identifying gaps in information. It was uniformly agreed that more scientifically-based studies are needed to determine the optimal conditions for each species of laboratory animals, including cage sizes, environmental enrichment, lighting, temperature, humidity, air changes, etc. There were differences of opinion among participants about changing guidelines and standards – some felt that public pressure forced change in the absence of data, relying more on expert opinion. Others felt that guidelines should only be changed when scientific data are available. In general, differences were largely geographical or cultural. Most participants agreed that guidelines must be beneficial to the animals and support good science. Many also agreed that guidelines are not productive or practical when they mandate specific conditions, but they should provide minimum standards. Data were presented showing that even if environmental conditions were standardized, there can be variability in experimental outcome due to differences in animal handlers. Other data showed that environmental enrichment can affect numerous anatomical and physiological parameters. This type of information shows that there are many unanswered questions regarding the scientific basis of animal care.

Discussion on Harmonization

The final session of the workshop was a discussion about harmonization of guidelines. Ten brief statements were made that supported harmonization or did not. rt it. Table 2 shows the participants in the panel. A key point of discussion was the definition of "harmonization." Distinctions were drawn between: harmonization vs. standardization; guidelines vs. regulation; performance vs. engineering standards.

Arguments against harmonization included the following:

- There are still too many gaps in the science to support harmonization.
- There are too many differences among countries – e.g., culture, tradition, values, laws, regulations, religious beliefs.
- It would pose limitations in the environmental range of experimental study. and in the process mask important biological effects

Points made in consideration of harmonization were:

- Consider harmonized practices rather than regulations – ethical review, animal care and use review, and national oversight authority.
- Process of harmonization should begin with exchange of opinions and thoughts.
- We should strive for harmonization of guidelines rather than standardization.
- Guidelines should:
- Provide clear benefits to the animals.
- Not interfere with research.
- Be based on science.
- Be published and used as reference tools.
- Flexibility should be allowed for:
- Best context-specific arrangements required to promote animal welfare and good science.
- Innovation to continuously challenge and increase standards.
- Flexibility permits changes in guidelines as more scientific evidence becomes available.
- Refer to the CIOMS Principles – International Guiding Principles for Biomedical Research Involving Animals (Council for International Organizations of Medical Sciences, 1985).

Future directions

Participants in the final discussion had some valuable suggestions for future steps. These included:

- Identify specific research problems that need addressing and organize an effort in multiple laboratories to generate publishable data – e.g., optimal enclosure dimensions, best caging material, environmental enrichment, euthanasia practices, and ventilation requirements.
- Form a consortium to coordinate research needs, funding and efforts in identified areas.
- Make an effort to collect data that have already been generated, but not reported.
- Encourage investigators to include more information about husbandry and experimental manipulations of animals in research papers.
- Make scientists more aware of the consequences of lab animal care.
- Harmonize education and training initiatives.
- Hold future meetings with fewer participants to continue and facilitate further dialogue, outline steps for future initiatives and agree on common goals.

The proceedings from this workshop are forthcoming. For information about their availability, check the ILAR website at www.dels.nas.edu/ilar.

Acknowledgements

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Table 1. Program Committee for the Workshop

Hilton Klein, Chair, Merck Research Laboratories, West Point, PA
Stephen Barthold, University of California, Davis
Coenraad Hendriksen, Netherlands Vaccine Institute
William Morton, Washington National Primate Research Center
Randall Nelson, University of Tennessee Medical School, Memphis
Emilie Rissman, University of Virginia Medical School
William Stokes, National Institute of Environmental Health Sciences, NIH

Table 2. Point/Counterpoint Panel Members

William Stokes, Moderator
John Crabbe – Oregon Health & Science University
Gilles Demers – ICLAS
Derek Forbes – FELASA
Nelson Garnett – OLAW, NIH
Clement Gauthier – CCAC
Naoko Kagiyama – Central Inst. For Exptl. Animals, Japan
Michael Kastello – Aventis Pharmaceuticals
Wim de Leeuw – Council of Europe
John Miller – AAALAC International
Jonathan Richmond – Home Office, UK

Harmonising veterinary care in Canada : CALAM/ACMAL Standards of Veterinary Care

Patricia V. Turner, Dept of Pathobiology, University of Guelph, Guelph, ON CANADA N1G 2W1

The Canadian Association for Laboratory Animal Medicine (CALAM/ACMAL) represents veterinarians working across Canada within the field of laboratory animal medicine. One of the key mandates of CALAM/ACMAL is to provide leadership for developing improved and humane methods of animal use in research, teaching, and testing in Canada. An important component for any strategy to refine research animal use is to ensure the quality and consistency of veterinary care that is provided to animals. In an effort to provide guidance in this area and to harmonize programs of veterinary care for animals used in teaching, testing and research in Canada, CALAM/ACMAL has recently issued a comprehensive statement on â€œStandards of Veterinary Careâ€ . Research institutions and regulatory bodies across Canada will use these new standards to formulate and evaluate appropriate veterinary care programs for laboratory animals in Canada.

The use of AAALAC International Accreditation Process to assure harmonisation in a multi-national company : a European approach

Margaret Landi (US), Timothy Morris (UK), John Brautigam (UK), Andrea Tamellini (Europe) GSK Pharmaceuticals, 709 Swedeland Road, UM 2620, King of Prussia, Pa. 19406, US

Current societies and cultures continue to seek ways to improve both the quantity and quality of life for humans and other animals. For those in biomedical research this privilege of performing animal based research is overseen and regulated by various national, regional or local laws. In turn, each institution where research is done generates policies and procedures governing the animal care and research program. At the time of a merger there can be wide diversity in practices involved with animal research; often due to differences in interpretation and implementation of differing laws.

At GSK we chose to use the Association for the Assessment and Accreditation for Laboratory Animal Care International (AAALAC Itl.) process as a method for the evaluation of the program of animal care and for harmonisation of policies and procedures. AAALAC-Itl. accreditation is a peer review of standards for all aspects of animal care. It does not duplicate other quality and validity ensuring systems such as GLP or FDA inspections, or national systems to ensure compliance with laws and regulations. The site visitors and the AAALAC Itl. Council focus on supporting the applicant in its aim to implement best practise in animal care, welfare and scientific procedures.

This talk will discuss the benefits and challenges to seeking AAALAC Itl. accreditation in a global company. The financial impact of AAALAC Itl. accreditation, the steps involved with instituting a new program, and the education of staff, investigators and administration will be discussed. Recent real life examples will be used from sites where AAALAC Itl. was a either new concept or where half of heritage programs had been previously AAALAC Itl. accredited. Also discussed will be working with AAALAC Itl. to create a uniform evaluation for one company with facilities in a number of different locations and countries.

AAALAC Itl. accreditation is the only globally applied process for confirmation of standards for laboratory animal care. It can help provide balance where there is a lack of international harmonisation. In a global Rorganisation it is a tool that can be applied as an important driver for world-wide implementation of best scientific practice in laboratory animal based research.

International and Interlaboratory Exchange : regulation and health Issues

Activities of the LASA Transport Working Group : New Guidelines for the Transport of Laboratory Animals

Jeremy Swallow, LASA Transport Working Group.

The presentation will give an update on the Guidance on the Transport of Laboratory Animals and the work of the LASA group.

March 2002 saw the inaugural meeting of the LASA Transport Working Group, almost 10 years since the publication of the first LASA/LABA Transport Guidelines. The participants were chosen from various UK organisations interested in the welfare of laboratory animals during transport. During that 10-year period there have been many changes to both national and EU legislation on the subject of animal transport. The advent of GM mice and globalisation has meant that more animals are transported internationally by air and as these journeys are by their nature complex it essential that the conditions of transport are correct to ensure their best welfare. These guidelines will reflect these changes giving more specific advice on journey planning, developing contingencies and species specific advice on the conditions of transport of the common laboratory species which has been expended to cover minipigs and amphibians.

Other initiatives the group have focussed on include: novel ways to calculate stocking densities in shipping crates, shipment tracking devices, the real incidence of deaths in transit, attempts to define the ideal bio exclusion material for shipping crates, a risk assessment of rabies in laboratory rodents, lobbying IATA, facilitating changes to legislation impacting EC directives, and UK import regulations for rodents and germplasm.

Involvement of Air France in international transportation of Laboratory Animals

Pierre Lamour - General Manager, Air France

Ever since the company Air France has carried animals intended for animal experimentation, it has had to face numerous actions from opponents, and much more scarcely, from supporters.

The start of the nineties was marked by a radicalism in opposition, mainly consisting of very strong communication campaigns, together with demonstrations and threats.

Confronted by these multiple attacks, many questions were raised in the different departments involved in the company. It is obvious that, at the time, especially between 1992 and 1995, there were strong internal hesitations on the subject. This can be easily understood, in so far as, for instance, the reception of demonstrators is not the main activity of an Air France agency!

Many factors, internal and external, helped to build up the company's global policy. Amongst these, the following have been decisive:

- Air France's knowledge of the animal protection NGO's functioning. This had been previously achieved through opposition to transport of other animal species,
- the implementation of a dialog between the company and the main French NGO's,
- the joint thoughts and actions of the research Ministry, the GIRCOR and the company *vis a vis* the NGOs, the European Council and the European Parliament,
- the internal creation of a specific department in charge of following up these files.

Notwithstanding Air France's present determination to achieve these transports, and for that matter not wishing to elaborate on the justification of animal experimentation, we wish to draw your attention to the negative consequences that any incident could induce.

Confident that nothing is definitively fixed, we request a total transparency in the handling of this file and the renewal of your support.

Factors affecting validity of health reports

Werner Nicklas, DipECLAM, German Cancer Research Centre Im Neuenheimer Feld 280 69120 Heidelberg, Germany

Health reports are important tools to aid the management of an animal facility and are frequently used as a basis for decisions. They are essential to evaluate the health status of animals, and a reliable health report is therefore of crucial importance to avoid the introduction of unwanted agents especially when genetically modified rodents are obtained from other experimental colonies. However, rechecking of animals upon arrival occasionally leads to disagreeing results which may be important if agents are detected for which animals are declared negative in the health report. FELASA has published recommendations in which general aspects for health monitoring (e.g., sample size, frequency of monitoring, agents to be monitored, format for health reports) are presented. Another FELASA recommendation is dealing with accreditation of diagnostic laboratories. However, these recommendations can only cover very general aspects. When establishing the monitoring programme, decisions on many details have to be made locally depending on specific needs or characteristics of the unit to be monitored. Factors that should be considered are the physical structure of the facility, species and strains of animals housed in the unit, and the procedures conducted including husbandry and clinical observation. It is therefore necessary that persons with specific expertise in laboratory animal medicine are involved to establish an appropriate monitoring programme so that it is tailored to specific needs. The diagnostic laboratory also plays an important role in the creation of health monitoring data. Qualification and experience of persons responsible for the laboratory as well as of those conducting the tests may have impact on the validity of results. In addition, results of bacterial identification or results from serological tests are frequently dependent on the methods or test kits used. It is a fact that disagreeing results may be obtained even if pure cultures of bacteria are identified by different laboratories. Ring tests show that not even important organisms such as *Streptococcus pneumoniae* or *Citrobacter rodentium* are properly identified by all laboratories involved in rodent health monitoring.

Rat Respiratory Virus : an Emerging Pathogen

Lela K. Riley, Robert S. Livingston, Research Animal Diagnostic Laboratory (RADIL)University of Missouri, Columbia, Missouri USA

Interstitial pneumonia of unknown etiology has been identified in laboratory rats. The disease appears to be widespread among laboratory rat colonies in the U.S. and Europe. Affected rats are typically asymptomatic, but occasionally exhibit coughing, sneezing and death following anesthesia. Histologic lesions are characterized by perivascular cuffs of mononuclear cells and interstitial pneumonia with infiltration of lymphocytes, macrophages and occasional neutrophils. Areas of hemorrhage are also seen. To identify the causative agent, lungs from affected rats were cultured on mammalian cell lines. Resulting cultures showed no cytopathic effect but were positive by immunofluorescence when probed with sera from affected rats, indicating growth of a virus. To determine if the in vitro propagated virus was the etiologic agent, groups of 4-5-week-old male rats were inoculated with in vitro propagated virus. Experimentally inoculated rats showed no clinical signs; however, rats seroconverted and at 8 weeks post-inoculation showed lymphoid perivascular mononuclear cuffing and interstitial pneumonia consistent with lesions observed in naturally infected rats. Based on these findings, the isolated virus is believed to be the etiologic agent and it has tentatively been designated Rat Respiratory Virus (RRV). Electron microscopic analysis of semi-purified RRV preparations indicated that the virus was 80-120 nm in diameter with short (510 nm) peplomers and was enveloped. Serologic assays have been developed and are being validated as diagnostic tools to determine the infectious status of rats.

Where written papers were not submitted abstracts only have been inserted

Development of an International Health Monitoring System (IHMS)

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Summary

International exchange of research rodents has heightened awareness of the need for a standardized health monitoring system to facilitate movement and safe introduction of animals between institutions. The FELASA Working Group has published recommendations to standardize testing systems within Europe. However equivalent guidelines have not been developed in the United States. Taconic has moved to develop and fully implement an International Health Monitoring System (IHMS) to periodically profile the health status of its rat and mouse colonies. The system was designed to essentially meet or exceed recommendations by the FELASA Working Group for the panels of agents monitored, sample sizes and frequency of testing for them in order to adequately profile the health status of breeding colonies and rodent cohort groups procured by animal users. Moreover, the reporting format was closely patterned on the format recommended by the Working Group to promote consistency in the way rodent health information is promulgated by testing laboratories and disseminated for review. For users of laboratory rodents submitting sample groups or sample materials to outside testing laboratories, the same panels may be used for the purpose of profiling the health status of shipment cohorts either to be sent to other institutions or following reception at the user's institution.

Introduction

Biomedical and pharmaceutical research has increasingly focused on harmonization of reagents, equipment and research standards to achieve international comparability of results and regulatory surveillance. This will introduce an approach to proactively include animal health surveillance as one of the main areas for harmonization of laboratory animal quality standards.

Several factors are important in this process. Firstly, the process for pharmacological, medical device and pharmaceutical product development and regulatory approval has changed substantially in the last few years. These changes relate to the multinational structure of most companies, which develop and test new products, process regulatory approvals and market on a global basis (Weisbroth and Poe, 2000). Drugs may be studied for efficacy in one country, tested for safety (toxicology) in another, undergo clinical trials somewhere else and have regulatory approvals processed simultaneously in every area in which it is intended to market the product. This requires harmonization of the parameters supporting these studies to achieve comparability in animal health, genetics, nutrition and of caging and animal care standards.

For the commercial breeder, this process has been the driver to respond to user requirements for reliable, adequate and timely health surveillance information. There has been a shift in recent years to genetically engineered rodents and rabbits. Hundreds of transgenic and mutant strains have been developed in many countries leading to a traffic in animals of uncertain health status being shipped around the world to collaborators, other breeders and institutional producers and scientific users. As appreciation of gene structure and function leads to correspondingly new areas of product development we should expect the global traffic in transgenic research rodents to continue. International exchange of research rodents has heightened awareness of the need for a standardized health monitoring system to facilitate movement and safe introduction of research animals between institutions. The user is faced with the problem of preserving the health status of his rodent facilities. The safe introduction of newly arriving rodents is dependent on health surveillance information that is adequately developed, current, representative of the breeding unit and presented in a comprehensible format. It has been already pointed out that there is no uniformity in the way that producers develop and present health surveillance information and such data as is provided is often unsatisfactory (Martin-Caballero *et al*., 2003).

The need to monitor laboratory animal health status is well established (Waggie, *et al*. 1994, Jacoby and Lindsey 1997, Weisbroth, *et al*. 1998, Kunstyr and Nicklas 2000, Shek and Gaertner 2002). Both the Institute of Laboratory Animal Resources (ILAR) *Guide* in the U.S. (ILAR 1996) and FELASA policy in Europe (Nicklas, *et al*. 2002) recommend a health surveillance based on microbial assessment as part of every properly managed animal care program. The scientific community in each of the main areas of concentration of biomedical research (the U.S., Europe, Japan and Korea) supports the concept of using research animals in terms of pathogen status by means of microbial assessment.

Microbial assessment has been defined as the science of evaluating representative sample groups from given production units against a specific listing of etiologic agents of disease to define the health status of animal residents in the source unit (Weisbroth, *et al.* 1998). The purpose of this is to detect and prevent introduction of disease agents, and to enable management and continuity of health maintenance programs at user institutions. Development of microbial assessment data forms the basis on which: 1) to establish and/or confirm the ongoing microbial status of commercial and institutional rodent production colonies, 2) to develop institutional procurement standards for supplier eligibility based on animal health criteria and 3) to continuously monitor the health status of research animals (Weisbroth, *et al.* 1998). The goal is to detect any pathogen from a specific list of infectious agents. The inference is made that if an agent is detected in the sample group, the larger group (i.e. the source colony) represented by the sample must be regarded as contaminated by the same pathogen(s). Of equal importance is the inability to detect any of the pathogens in the profile because designated production units may be demonstrated as free from the specific agents listed in the profile on the assumption of valid detection methodology and adequate

representation by the sample group. Scheduled, repetitive testing provides current information for continuously updating the health status of closed production or user units to note either changes that have occurred (ingress of contamination) or to objectively confirm that there have been no changes.

There has been some variation in the way animal health experts have shaped health surveillance programs. Variation can be noted in their recommendations regarding sample sizes, periodicity of sampling schedules, in whether there is recognition that some agents are more prevalent than others, in recognizing that some agents are more pathogenic than others and in the profiles or lists of pathogenic agents regarded as important for monitoring. Both Taconic in the U.S. and M&B in Denmark, had separately developed health surveillance programs and these were shaped to meet production and user expectations.

In an effort to address international standards for rodent quality, AALAS created a Scientific Advisory Committee in 1999. A Microbiological Monitoring Group was formed with international representation and charged to "to develop health assessment and reporting formats that allow efficient international movement of laboratory rats and mice" (Smith 2000). Since health assessment resolves itself to testing data derived from sample animals against specified lists of pathogens, the Group grappled with the issue of how extensive the lists should be. It was unable to achieve consensus on this issue and disbanded after two years without resolution or recommendation. In the U.S., there is, at present, no expert committee process equivalent to the FELASA Working Group and its published recommendations.

In Europe, FELASA has defined health surveillance parameters for constituent members. These recommendations for both users and producers were developed by expert Working Groups and first published in 1994 and 1996 (Kraft *et al*. 1994, Rehbinder *et al*., 1996), and updated in the revision of 2002 (Nicklas *et al.* 2002). It was in this climate that M&B conducted its own health surveillance program in compliance with current FELASA recommendations. Following the merger of Taconic and M&B in 2002, the question for Taconic was whether to continue these separate programs or to harmonize health surveillance across the U.S. and Europe. The decision was made to develop a single, harmonized health surveillance program to meet the needs of a global production and client base. To accomplish this task, the Taconic health surveillance program and the FELASA recommendations were harmonized, named the International Health Monitoring System (IHMS) and implemented in June, 2003 (Europe) and January 2004 (USA). The guidelines for the harmonization process were as follows:

1) Because of the heightened prevalence of certain viral and bacterial infections (e.g., MHV, the parvoviruses) compared to others, it was desired to retain Taconic's higher frequency of testing for them than the quarterly schedule for such agents recommended by FELASA.
2) FELASA recommended testing for certain infrequent viral (e.g. Ectromelia) and bacterial agents (e.g. *Clostridium piliformi*), more frequently than the Taconic program. It was agreed that FELASA guidelines for frequency of testing, wherever they exceeded Taconic USA's, would be complied with, and conversely, where the Taconic USA program had a higher frequency of testing, that schedule would be continued in the IHMS program. The result was that in many instances, the IHMS program has a higher frequency of testing than FELASA guidelines.
3) There were certain agents recommended for monitoring by FELASA (e.g. *Klossiella* and *Bordetella bronchiseptica*), but not by Taconic, and others required by Taconic (e.g., Cilia-Associated Respiratory Bacillus, LDHV, Mouse Thymic Virus, Polyoma and K viruses) but not by FELASA. The decision was made to retain all agents required by either program.
4) There was common agreement on which agents to test for in both rats and mice, compared to those monitored in a single host species. Wherever this was discrepant, e.g. the Mouse adenoviruses, it was decided to monitor both host species.
5) The Taconic USA program routinely included histopathology of key organs, whereas FELASA recommended histopathology only in the presence of suspicious clinical signs or gross lesions. Because of the added insight to animal health afforded by routine histopathology, it was retained.
6) FELASA recommendations (except for very small units) are inflexibly based for most common agents on 10 samples per quarter or 40 per year per unit being monitored. Taconic's, on the other hand, were flexible and more influenced by the size of the breeding unit and an agent by agent assessment of the level of concern about prevalence in the field or potential damage of a contamination for users. At a minimum, the 40 tests per year recommended by FELASA were continued in the IHMS program. In fact, the test frequency for most target organisms exceeds the minimum recommendation.
7) The Taconic reporting format had both narrative descriptions and tabular listings of findings. One feature of the narrative section was a historical recap of significant findings, if any, since inception of the breeding unit. The FELASA format, on the other hand, was entirely tabular with listing of historical data limited to the last 6 quarters (18 months). It was decided to structure the IHMS findings as a tabular report as recommended by FELASA, but to extend the historical recap to a forward rolling last 24-36 months.
8) There were, initially, some differences between the laboratories used by Taconic USA and M&B in the methods used to detect the various agents. For example, Taconic used PCR as the primary screening tool for detection of the Tyzzer's Disease agent, *Clostridium piliforme*, whereas M&B used ELISA serology, as is more common in Europe. The goal, now achieved, was to harmonize test methods between Taconic's diagnostic laboratories, as well as the testing regimen.

Structure and Schedule of the IHMS

The IHMS consists of a biweekly sample submission schedule of either serum samples, sample groups of animals, or both. In the test year for the unit, there will be 26 separate occasions on which samples are submitted for testing. The sample number and the specific tests to be conducted are determined from a schedule drawn up in advance that takes into consideration all of the points 1-8 above. The panel types and frequency are summarized in Table 1 and their annual deployment for a breeding unit schedule in Table 2.

The panels are graded such that each succeeding panel adds agents to the list of those submitted more frequently.

This approach is in accord with the "tiered" or "smart testing" regimen recommended by Laber-Laird and Proctor (1993). The numeric task lay in balancing the frequency for each type panel to achieve more frequent testing for the agents of greatest concern but economically not overtesting for infrequent or rare agents. At the same time, the program needed to adhere to the tests per year per unit for each agent recommended by FELASA. The IHMS-2 panel is outlined in Table 3, and consists of a serologic screen of the viral agents of greatest prevalence for both rodent species. Table 4 is an outline of the IHMS-6 panel. All the agents in the IHMS-2 panel are incorporated to augment the IHMS-6 panel. Similarly, the IHMS-13 panel (Table 5) incorporates the IHMS-6 panel, IHMS-26 (Table 6) the IHMS-13 panel and IHMS-52 (table 7) the IHMS-26 panel.

Sample lots of test sera and/or animals are transferred to in-house diagnostic laboratories in Laven, Denmark for Taconic Europe operations and to Rockville, MD for Taconic USA. These two laboratories conduct all diagnostic necropsy, sample collection and testing, data compilation and reporting.

Diagnostic processing begins on the day of arrival or the next day and is initiated by a process that records background and case history, sets up data collection forms and assigns a unique Accession Number (Acc. No.) for tracking each sample lot. Animals are delivered to the necropsy lab from a staging area; described in detail elsewhere (Weisbroth *et al*. 1998).

All results are accumulated either in hard copy or electronically in a single folder identified by the Acc. No. Uncertain or unusual results may be referred to any of several institutional or commercial reference laboratories for confirmation or resolution of problematic in-house results. When all the tests have been completed they are sent to a data center for entry into the database. These results are available on line for public review and download. Table 8 represents a downloaded health report from one of the Taconic M&B production units. In accord with FELASA recommendations for reporting format (Nicklas *et al*. 2002), the following points pertaining to the IHMS Animal Health Report should be noted:

1. The top of the page information identifying the breeding unit (barrier) location, species and strains housed in the unit and date of issue all comply with the recommended FELASA format.
2. The column headings for target organisms, test frequency, test method, latest test date, latest test results, identification of the laboratory conducting the tests and historical (cumulative) results, similarly are in accord with the recommended FELASA format.
3. The test methods and other abbreviations recommended by FELASA are complied with in the Taconic IHMS format.
4. Diagnostic results data in the Taconic IHMS format are expressed as the number positive/number tested as recommended by FELASA.

There are several differences in the Taconic IHMS format that essentially expand on the recommended FELASA format. These additions are summarized as follows:

1. To more accurately represent the health status of the particular breeding unit, the latest test date is defined as the date the sample lots of animals are removed from the barrier for testing and not as the date the health report is issued (which may be 2-3 weeks later).
2. Commonly used acronyms for target organisms are indicated, where appropriate
3. Health reports are meant to define the microbial status of animals within the designated location. Since breeding barriers, for reasons of efficiency may contain both or either rodent species, the target organism listing includes all agents in the panels for both rats and mice. This reporting format, used for consistency, also serves to inform which target organisms are not tested for, and are therefore of unknown status in the reporting unit. The species actually tested for each agent is indicated in a separate column for that purpose.
4. Breeding barrier units may consist of single or multiple rooms. Accordingly, the barrier is referred to as a Health Reporting Group (HRG) since for health purposes, it must be assumed that with the open caging system used by Taconic, a communicable health condition could probably not be confined to a single room. Both the HRG and the location (room designation) within are noted in the top of the page information.
5. The original source colony at Taconic that supplied each of the strains for breeding in the HRG barrier are noted in the top of the page information.
6. The historical results are actually cumulative results and meant to summarize a forward rolling 24-36 months as each latest test result is added to the total. It would be difficult to overstate the importance of cumulated results of repetitive test periods on strengthening the statistical confidence of continued negative results in the closed breeding populations represented by the HRG.

Conclusion

A cautionary note should be added about the significance that breeders and users should attach to health reports based on the FELASA Working Group recommendations. There is a tendency on the part of the public, which should be resisted, to view the Health Reports as representing absolute lists of agents from which good quality research rodents should be free. Rather, the expert Working Group assembled the panels on the basis that the included agents represented those organisms whose presence or absence should be monitored by diagnostic surveillance in both breeder and user colonies because of their potential to adversely affect rodent health or biologic response. The panels were not made to represent an "all or none" standard of acceptability. The concept was that a rodent population could be determined as acceptable or not, depending on individual user requirements when provided with such information. Words such as "required" and "compliance" were avoided, and words such as "recommendation" and "in accordance with" were used. The Working Group stressed the point that results of such testing should be interpreted by individuals knowledgeable in rodent health such as Category D specialists (Nevalainen *et al*. 1999) and not be simply used as a procurement specification. In that spirit, we have presented a practical implementation of FELASA's recommendations for microbiological assessment programs and dissemination of health status information to the scientific community.

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Table 1.
Deployment schedule of IHMS panels for a breeding unit

Panel Designation	Interval Schedule (in weeks)	Panels per year
IHMS-2	2	18
IHMS-6	6-7	4
IHMS-13	12-14	2
IHMS-26	26	1
IHMS-52	52	1
Total		26

Table 2

One year test schedule for a breeding unit

Week No.	Panel Employed	Week No.	Panel Employed
2	IHMS-2	28	IHMS-2
4	IHMS-2	30	IHMS-2
6	IHMS-6	32	IHMS-6
8	IHMS-2	34	IHMS-2
10	IHMS-2	36	IHMS-2
12	IHMS-13	38	IHMS-13
14	IHMS-2	40	IHMS-2
16	IHMS-2	42	IHMS-2
18	IHMS-2	44	IHMS-2
20	IHMS-6	46	IHMS-6
22	IHMS-2	48	IHMS-2
24	IHMS-2	50	IHMS-2
26	IHMS-26	52	IHMS-52

Table 3

Structure of the IHMS-2 Panel: Serology
Schedule: Performed every 2 weeks

Agents Detected (Acronym)	Species tested	Primary method	Alternate method
Mouse hepatitis virus ((MHV)	Mouse	ELISA	IFA
Mouse minute virus (MMV)	Mouse	ELISA	HAI
Mouse parvovirus (MPV)	Mouse	ELISA	IFA
Mouse rotavirus (EDIM)	Mouse	ELISA	IFA
Kilham's rat virus (KRV)	Rat	ELISA	HAI
Rat parvovirus (RPV)	Rat	ELISA	HAI
Toolan's H-1 virus (TH1)	Rat	ELISA	HAI
Rat coronavirus (RCV)	Rat	ELISA	IFA
Sialodacryoadentitis virus (SDAV)	Rat	ELISA	IFA

Table 4

Structure of the IHMS-6 Panel: Serologic and diagnostic tests in addition to the IHMS-2 panel
Schedule: Performed every 6 weeks

Agents Detected (Acronym)	Species tested	Sample type	Primary method	Alternate method
Viruses				
Encephalomyelitis virus (GD7)	M, R	serum	ELISA	IFA
Pneumonia virus of mice (PVM)	M, R	serum	ELISA	IFA
Sendai virus (SEN)	M, R	serum	ELISA	IFA
Bacteria, Fungi				
Mycoplasma sp.	M, R	serum	ELISA	IFA
Clostridium piliforme	M, R	feces	PCR	serology
Salmonella sp.	M, R	feces	culture	
Citrobacter rodentium	M	feces	culture	
Pseudomonas aeruginosa	M, R	oropharyngeal swab	culture	
Klebsiella sp.	M, R	oropharyngeal swab	culture	
Streptococcus, B-hemolytic	M, R	oropharyngeal swab	culture	
Streptobacillus moniliformis	M, R	oropharyngeal swab	culture	
Streptococcus pneumoniae	R	nasopharyngeal swab	culture	
Corynebacterium kutscheri	M, R	nasopharyngeal swab	culture	
Pasteurella sp.	M, R	nasopharyngeal swab	culture	
Bordetella bronchiseptica	M, R	nasopharyngeal swab	culture	
Staphylococcus aureus	M, R	nasopharyngeal swab	culture	
Arthropod ectoparasites	M, R	skin surface	direct microscopy	
Enteric helminths (pinworms)	M, R	cecum	direct microscopy	
Enteric flagellates	M, R	ileum	direct microscopy	
Klossiella muris	M,R	kidney	histopathology	
Trichosomoides crassicauda	R	urocyst	direct microscopy	
Eimeria sp., other helminthes	M, R	feces	fecal flotation	

Table 5

Structure of the IHMS-13 Panel: Serologic and diagnostic tests in addition to the IHMS-6 panel
Schedule: Performed every 13 weeks

Agents Detected (Acronym)	Species tested	Sample type	Primary method	Alternate method	
Viruses					
Lymphocytic choriomeningitis (LCMV)		M	serum	ELISA	IFA
Bacteria, Fungi					
Cilia-associated respiratory bacillus (CARB)	M, R	serum	ELISA	IFA	
Corynebacterium bovis*	M, R	skin	culture		
Helicobacter sp.	M, R	feces	PCR		
Histopathology	M, R	key organs (liver, lung, kidney, ileum)	microscopy		

* immunodeficient strains only

Table 6

Structure of the IHMS-26 Panel: Serologic and diagnostic tests in addition to the IHMS-13 panel
Schedule: Performed every 26 weeks

Agents Detected (Acronym)	Species tested	Sample type	Primary method	Alternate method
Viruses				
Ectromelia	M	serum	ELISA	IFA
Hantaan virus	M, R	serum	ELISA	IFA
Mouse adenovirus (Mav 1 or FL)	M, R	serum	ELISA	IFA
Mouse adenovirus (Mav 2 or K87)	M, R	serum	ELISA	IFA
Mouse cytomegalovirus (MCMV)	M	serum	ELISA	IFA
Respiratory enteric virus III (REO3)	M, R	serum	ELISA	IFA

Table 7

Structure of the IHMS-52 Panel: Serologic and diagnostic tests in addition to the IHMS-26 panel
Schedule: Performed every 52 weeks

Agents Detected (Acronym)	Species tested	Sample type	Primary method	Alternate method
Viruses				
K virus (KV)	M	serum	ELISA	HAI
Lactic dehydrogenase elevating virus (LDHV)	M, R	serum	CHEM	
Polyoma virus (POLY)	M	serum	ELISA	IFA
Thymic virus	M	serum	IFA	
Bacteria, Fungi				
Clostridium piliforme (CPIL)	M, R	serum	IFA	ELISA
Corynebacterium kutscheri (CKUT)	M, R	serum	IFA	ELISA
Encephalitozoon cuniculi (ECUN)	M, R	serum	ELISA	HAI
Pneumocystis carinii	M,R	lung	PCR	
Hemobartonella muris	M	stained blood film	microscopy	
Eperythrozoon coccoides	M	stained blood film	microscopy	

Table 8

Taconic Health Reports

Quality Laboratory Animals and Services for Research

Production Site:	Tornbjerg, Denmark	**Date of Issue:**	Apr/22/2004
Health Report Group (HRG):	EBU150	**Species and # Lines Present:**	Mice: 1 Line
Locations in HRG:	E15001		Rats: 3 Lines
Date Location First Occupied:	Feb/10/2003	**Transferred From:**	E12501, E12701, Gnotobiotics Center
Taconic Health Standard:	Murine Pathogen Free	**Animal Lines:**	BB, LEWIS, MREN2, SDMOL

Latest Test Date is the date animals are removed from the colony to begin the health testing process.

Compilation of all results for web posting may take several weeks.

Target Organism	Test Frequency	Testing Laboratory	Test Method	Species Tested M =Mice R = Rats	Latest Test Date	Latest Result	2004 Cumulative Results	2003 Results	2002 Results
Viruses									
Mouse Hepatitis Virus (MHV)	2 weeks		ELISA	M	-	-	-	-	-
Mouse Minute [parvo] Virus (MMV)	2 weeks		ELISA	M	-	-	-	-	-
Mouse Parvovirus (MPV)	2 weeks		ELISA	M	-	-	-	-	-
Mouse Rotavirus (EDIM)	2 weeks		ELISA	M	-	-	-	-	-
Kilham's Rat Virus (KRV)	2 weeks	Taconic M&B	ELISA	R	Mar/22/2004	0/2	0/26	0/151	-
Rat Coronavirus (RCV)	2 weeks	Taconic M&B	ELISA	R	Mar/22/2004	0/2	0/26	0/55	-
Rat Parvovirus (RPV)	2 weeks	Taconic M&B	ELISA	R	Mar/22/2004	0/2	0/26	0/151	-
Sialodacryoadenitis Virus (SDAV)	2 weeks	Taconic M&B	ELISA	R	Mar/22/2004	0/2	0/26	0/55	-
Toolan's H-1 Parvovirus (TH1)	2 weeks	Taconic M&B	ELISA	R	Mar/22/2004	0/2	0/26	0/151	-
Encephalomyelitis Virus (GD7)	6 weeks	Taconic M&B	ELISA	M, R	Jan/13/2004	0/5	0/10	0/20	-
Pneumonia Virus of Mice (PVM)	6 weeks	Taconic M&B	ELISA	M, R	Jan/13/2004	0/5	0/10	0/35	-
Sendai Virus	6 weeks	Taconic M&B	ELISA	M, R	Jan/13/2004	0/5	0/10	0/35	-
Lymphocytic Choriomeningitis Virus (LCM)	13 weeks	Taconic M&B	ELISA	M	Oct/21/2003	0/5	-	0/30	-
Ectromelia Virus	26 weeks		ELISA	M	-	-	-	-	-
Hantaan Virus	26 weeks	Taconic M&B	ELISA	M, R	Oct/21/2003	0/5	-	0/20	-
Mouse Adenovirus (FL) (MAV1)	26 weeks	Taconic M&B	ELISA	M, R	Oct/21/2003	0/5	-	0/20	-
Mouse Adenovirus (K87) (MAV2)	26 weeks	Taconic M&B	ELISA	M, R	Oct/21/2003	0/5	-	0/20	-
Mouse Cytomegalovirus (MCMV)	26 weeks		ELISA	M	-	-	-	-	-
Respiratory Enteric Virus III (REO3)	26 weeks	Taconic M&B	ELISA	M, R	Oct/21/2003	0/5	-	0/20	-
K Virus	52 weeks		ELISA	M	-	-	-	-	-
Lactic Dehydrogenase Elevating Virus (LDHV)	52 weeks		CHEM	M	-	-	-	-	-
Polyoma Virus	52 weeks		ELISA	M	-	-	-	-	-
Thymic Virus	52 weeks		IFA	M	-	-	-	-	-
Bacteria, Mycoplasma, Fungi									
Beta hemolytic Streptococcus	6 weeks	Taconic M&B	CULT	M, R	Jan/13/2004	0/5	0/10	0/35	-
Bordetella bronchiseptica	6 weeks	Taconic M&B	CULT	M, R	Jan/13/2004	0/5	0/10	0/35	-
Citrobacter rodentium	6 weeks	Taconic M&B	CULT	M	Oct/21/2003	0/5	-	0/35	-
Clostridium piliforme	6 weeks	Taconic M&B	PCR	M, R	Jan/13/2004	0/5	0/10	0/173	-
Corynebacterium kutscheri	6 weeks	Taconic M&B	CULT	M, R	Jan/13/2004	0/5	0/10	-	-
Klebsiella oxytoca	6 weeks	Taconic M&B	CULT	M, R	Jan/13/2004	0/5	0/10	Mar-35	-
Klebsiella pneumoniae	6 weeks	Taconic M&B	CULT	M, R	Jan/13/2004	0/5	10-Jan	22/35	-
Mycoplasma sp.	6 weeks	Taconic M&B	ELISA	M, R	Jan/13/2004	0/5	0/10	0/35	-
Pasteurella pneumotropica	6 weeks	Taconic M&B	CULT	M, R	Jan/13/2004	0/5	0/10	0/35	-
Other Pasteurella sp.	6 weeks	Taconic M&B	CULT	M, R	Jan/13/2004	0/5	0/10	0/35	-
Pseudomonas aeruginosa	6 weeks	Taconic M&B	CULT	M, R	Jan/13/2004	0/5	0/10	0/35	-
Salmonella sp.	6 weeks	Taconic M&B	CULT	M, R	Jan/13/2004	0/5	0/10	0/35	-
Staphylococcus aureus	6 weeks	Taconic M&B	CULT	M, R	Jan/13/2004	5-Jan	10-Feb	Aug-35	-
Streptobacillus moniliformis	6 weeks	Taconic M&B	CULT	M, R	Jan/13/2004	0/5	0/10	0/35	-
Streptococcus pneumoniae	6 weeks	Taconic M&B	CULT	M, R	Jan/13/2004	0/5	0/10	0/35	-
Cilia Associated Respiratory Bacillus (CARB)	13 weeks	Taconic M&B	ELISA	M, R	Dec/02/2003	0/5	0/5	0/35	-
Corynebacterium bovis **	13 weeks		CULT	M, R	-	-	-	-	-
Helicobacter hepaticus & H. billis	13 weeks	Taconic M&B	PCR	M, R	Dec/02/2003	0/5	0/5	0/193	-
Other Helicobacter sp.	13 weeks	Taconic M&B	PCR	M, R	Dec/02/2003	0/5	0/5	0/193	-
Clostridium piliforme	52 weeks		IFA	M, R	-	-	-	-	-
Corynebacterium kutscheri	52 weeks	Taconic M&B	IFA	M, R	Oct/21/2003	0/5	-	0/20	-

Pneumocystis carinii	52 weeks	Taconic M&B	PCR	M, R	Oct/21/2003	0/5	-	0/20	-
Parasites									
Aspiculuris sp.	6 weeks	Taconic M&B	MICR	M, R	Jan/13/2004	0/5	0/10	0/35	-
Eimeria sp.	6 weeks	Taconic M&B	FLOT	M, R	Jan/13/2004	0/5	0/10	0/35	-
Entamoeba muris	6 weeks	Taconic M&B	MICR	M, R	Jan/13/2004	0/5	0/10	-	-
Giardia muris	6 weeks	Taconic M&B	MICR	M, R	Jan/13/2004	0/5	0/10	-	-
Hymenolepis sp.	6 weeks	Taconic M&B	FLOT	M, R	Jan/13/2004	0/5	0/10	0/35	-
Klossiella muris	6 weeks	Taconic Anmed	HIST	M, R	Jan/13/2004	0/5	0/10	-	-
Liponyssus sp.	6 weeks	Taconic M&B	MICR	M, R	Jan/13/2004	0/5	0/10	0/35	-
Myobia sp.	6 weeks	Taconic M&B	MICR	M, R	Jan/13/2004	0/5	0/10	0/35	-
Myocoptes sp.	6 weeks	Taconic M&B	MICR	M, R	Jan/13/2004	0/5	0/10	0/35	-
Notoedres sp.	6 weeks	Taconic M&B	MICR	M, R	Jan/13/2004	0/5	0/10	0/35	-
Polyplax sp.	6 weeks	Taconic M&B	MICR	M, R	Jan/13/2004	0/5	0/10	0/35	-
Psorergates sp.	6 weeks	Taconic M&B	MICR	M, R	Jan/13/2004	0/5	0/10	0/35	-
Radfordia sp.	6 weeks	Taconic M&B	MICR	M, R	Jan/13/2004	0/5	0/10	0/35	-
Spironucleus sp.	6 weeks	Taconic M&B	MICR	M, R	Jan/13/2004	0/5	0/10	-	-
Syphacia sp.	6 weeks	Taconic M&B	MICR	M, R	Jan/13/2004	0/5	0/10	0/35	-
Trichomonads	6 weeks	Taconic M&B	MICR	M, R	Jan/13/2004	0/5	0/10	0/35	-
Trichosomoides crassicauda	6 weeks	Taconic M&B	MICR	R	Jan/13/2004	0/5	0/10	0/30	-
Encephalitozoon cuniculi	52 weeks	Taconic M&B	ELISA	M, R	Oct/21/2003	0/5	-	0/35	-
Eperythrozoon coccoides	52 weeks		MICR	M	-	-	-	-	-
Hemobartonella muris	52 weeks		MICR	M	-	-	-	-	-
Histopathology									
Ileum	13 weeks	Taconic Anmed	HIST	M, R	Dec/02/2003	0/5	0/5	0/30	-
Kidney	13 weeks	Taconic Anmed	HIST	M, R	Dec/02/2003	0/5	0/5	0/30	-
Liver	13 weeks	Taconic Anmed	HIST	M, R	Dec/02/2003	0/5	0/5	0/30	-
Lung	13 weeks	Taconic Anmed	HIST	M, R	Dec/02/2003	0/5	0/5	3/30 f	-
Other	N/A		N/A	M, R	-	-	-	-	-
Gross Pathology									
Middle Ear Exam	6 weeks	Taconic M&B	N/A	M, R	Jan/13/2004	0/5	0/10	0/35	-
Necropsy Findings	6 weeks	Taconic M&B	N/A	M, R	Jan/13/2004	0/5	0/10	0/35	-

** *Corynebacterium bovis* is tested in immunodeficient (mice with scid or nude mutation; rats with nude mutation) animals only

a. Rats Only

b. Chronic Nephrosis

c. Mineralization

d. Nephritis and/or Tubular Necrosis

e. Focal Hepatitis

f. Perivascular Lymphoid Aggregates

g. Pneumonitis

h. Tumor

i. *Helicobacter bilis* (only)

j. *Helicobacter hepaticus* (only)

k. *Helicobacter bilis* & *hepaticus*

Approved By:

James G Geistfeld DVM, MBA, Dip. ACLAM

Evaluation of the efficacy of antibiotic treatment and cross-fostering for elimination of the *Helicobacter* from naturally infected Mice colonies

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Murine Helicobacter species have been shown to be efficient colonizers of the cecum and colon.

H. hepaticus causes persistent hepatitis and hepatocellular carcinoma in certain strains of mice and has been associated with spontaneous proliferative colitis in immunodeficient mice and monoinfected germfree mice. For these reasons, it has become important to develop methods to eradicate Helicobacter spp. from naturally infected colonies. While embryo rederivation is effective in eliminating *Helicobacter ssp*., it is costly, labor-intensive, and requires special equipment. This method was proposed as a reliable, cost-effective alternative. One Helicobacter tablet (BioservR), consisting metronidazole, ampicillin, and bismuth was given once a day to the infected pregnant female (confirmed by PCR), starting on the 10th day after the plug was seen and until pups were born. A foster mother was time-mated to deliver less than 24 hours after the donor (but could be up to 72 hours before). Treated litters were fostered to a Helicobacter negative mother within 24 hours of being born. Fostered pups were tested by PCR on fecal pellets at 4 and 8 weeks of age. Using this method, we rederived 161 litters from 38 different stains. Out of these litters, only three (2%) came back positive for *Helicobacter spp*. at either 4 or 8 weeks of age. Of these 3 litters, 2 were from the same strain and one was cross fostered at the limit of our 24-hour cut-off line after birth. After 6-18 months post-rederivation, the colonies are still negative by PCR on fecal pellets from sentinel cages. This procedure revealed to be highly effective to eradicate *Helicobacter spp*. from mouse colonies.

Combining Good Science and Animal Welfare

Ethical Review Processes in Europe : A FELASA Working Group Study

Jane Smith

The FELASA Working Group on ethical evaluation of animal experimentation is charged with describing "practical guidelines on how a responsible ethical evaluation is performed". The Working Group has the following members: Jane Smith (Convener); Frank van den Broek; Jordi Canto; Hansjoachim Hackbarth; Osvaldas Ruksenas; and Walter Zeller. Further information can be found at: www.felasa.org/working/index.html

The Group began its work by using a questionnaire to gather information on how each of the various countries represented in FELASA currently approaches ethical review of laboratory animal use in practice. The responses to the questionnaire and Working Party discussions suggest that, although local practices differ, there is an emerging consensus on the key elements that any ethical review process should involve.

Drawing on the findings of the questionnaire, this presentation will describe and explore general principles for ethical review in practice. This will include consideration of legal requirements; the scope of work reviewed and the 'level' at which review is approached; who is involved and how the process is organised; the factors considered in the review; needs for on-going review after initial permission is granted; wider impacts of the review process; transparency and openness.

This study will be published on the FELASA WebPage in 2005: www.felasa.org

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Strategies for effective IACU communication and how to facilitate the Protocol Review Process

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To many investigators, the Animal Study Proposal (ASP) review process is cumbersome and unyielding. The process often takes several months from the time of initial writing until final protocol approval. The frustrated investigator often wishes that the Institutional Animal Care and Use Committee (IACUC) would communicate the rules and regulations more effectively so that all of the necessary requirements or protocol modifications could be completed quickly and the research could begin without delay. The IACUC of the National Heart, Lung and Blood Institute (NHLBI) has continued to respond to investigator concerns and modified the ASP review process accordingly. A web-based protocol submission process and a novel IACUC meeting structure was designed to facilitate the ASP process from start to finish. This presentation will describe our ASP review and management process as well as our Intranet Animal Study Proposal (IASP) on-line protocol submission process. The IASP facilitates completeness of forms, links to reference material, sends reminders for renewals and annual reviews, stores training information for personnel, and is available 24-hours a day/7 days a week.

Nordic Forum for Ethical evaluation of Animal experiments

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Summary

Ethical evaluation of animal experiments is gaining an increasingly important role in the general review process of animal experiments. In order to discuss various aspects of ethical evaluation, a workshop was organised in Helsinki. Forty participants representing the scientific community, animal welfare organisations and regulators from Nordic and Baltic countries and The Netherlands took part. During the workshop, a scheme for a cost-benefit analysis was developed and discussed. Costs refer to the costs the animals pay when they are used in experiments, such as pain, suffering and distress. Benefits are defined as the benefits gained by humans or other target groups resulting from animal studies. A third dimension for the evaluation was introduced: the means or cost modifiers. With the help of the means, the costs to the animals can be decreased. The cost-means-benefit model was tested in practice by evaluating experimental protocols.

1. Introduction

Ethical evaluation of animal experiments is gaining an increasingly important role in the general review process of animal experiments and there has been overall interest to improve the evaluation process. Furthermore, a cost-benefit analysis is likely to become mandatory in the revised EU directive. Many countries have already included the evaluation in the review process, but no commonly accepted method is available. In order to discuss the process and the factors contributing to the analysis, a Workshop on the cost-benefit principle for ethical evaluation of animal experiments was organised in Helsinki during 7-9 November 2003. Altogether 40 participants from Denmark, Estonia, Finland, Iceland, Latvia, Lithuania, Norway, Sweden and The Netherlands representing the (laboratory animal) scientific community, pharmaceutical industry, regulators and animal welfare organisations were invited. The organising body was the Cooperation Group for Laboratory Animal Sciences within the Finnish Ministry of Education and funding was received from the Academy of Finland, the Finnish Ministry of Education, the Finnish Ministry of Agriculture and Forestry, the NOVA University and the Finnish Society for the Protection of Animals. The overall purpose was to search for new tools in the ethical evaluation to result in an ethically sustainable, scientifically sound and transparent review.

2. Working Process

After introductory lectures and a review of existing evaluation schemes, working groups defined costs and benefits: costs refer to the costs the animals pay when used in experiments, such as pain, suffering and distress. Benefits refer to the benefits humans are expected to receive from animal experiments, e.g. improved therapies for human diseases or increased knowledge from basic science studies. Attempts were made to see how benefits and costs could be weighed at the same time. An experiment should be considered ethically acceptable when the benefits outweigh the costs.

In existing scoring systems, factors like animal species and number, animal source, experimental design and procedures are scored. This mathematical scoring approach was not supported by the participants, as this gives a false impression of objective accuracy. Instead classification of costs versus benefits into three classes was considered as the most suitable analysis method, as first published by Bateson (1986). If the costs to the animals are severe, and the expected benefits low, the outcome of the ethical evaluation will be rejection. When costs are high-medium, and benefits medium-low, chances for rejection exist. A third dimension for the evaluation was introduced: the means or cost modifiers. When it is possible to execute a study with a refined technique that will reduce suffering, and thus costs, chances for a positive outcome from the ethical evaluation will increase. The factors that should be evaluated as parts of costs, benefits and means are shown in Table 1. The use of means is encouraged to decrease the costs to the animals at all times, see Figure 1.

3. Cost-Means-Benefit Evaluation

The cost-means-benefit model was tested in practice by evaluating two applications sent to a regional ethics committee in Sweden. The two protocols were presented to the audience, where after each participant made the ethical evaluation anonymously by putting their evaluation into the Bateson chart.

The first project studied new gene therapy for a hereditary human disease, based on a gene defect resulting in kidney disorder (Alperts disease). About 4 % of these human patients require dialysis. The disease is painful for the patients and treatment is expensive for society. A mongrel dog colony in USA exhibits a similar disease to Alperts disease, and therefore this provides a good model to be used in this study. The costs to the animals are long transportation distance, surgical operation taking three hours and a kidney biopsy every third week under anaesthesia. The risk of collapse of kidney function is quite small, as has been shown in a previous study. In case of successful treatment, i.e. without the induction of side effects, the dogs can survive to an old age without problems. The results of the individual evaluations are shown in Figure 2.

In the second case study, a knock-out mouse model was used for studying a gene's role in the development of anaemia and formation of red blood cells. Diamond-Blackfan anaemia is a serious genetic disease in young children leading to skeletal disorders, retarded growth and heart dysfunctions. Without therapy, the patients will die within two to ten years. The disease is rare, about one child out of 200 000 born suffers from this disease. The aim was to investigate the ability of the mice to produce new blood cells as a response to an induced anaemia. The costs to the mice were induction of anaemia, seven blood samples taken and being housed singly. The results of the individual evaluation are shown in Figure 2.

In both case studies and especially in the study 2, the results showed quite high variation in the scoring of the degree of costs and the importance of the benefits. The difficulties in the weighing process are that costs and benefits are scored subjectively. The benefits seem to be more influenced by subjective evaluation than the costs; these can be based on more objective criteria and are more concrete than the benefits.

4. Conclusions

An experiment can be considered as ethically justifiable when the benefits are rated higher than the costs. Mathematical scoring systems are not the best option, because they give a false impression of objective accuracy. The two-dimensional Bateson scheme was proposed as representing the optimal basis of cost-benefit analyses. With the help of a new element, the means or cost modifiers, the costs to the animals can be decreased. By decreasing the costs to the animals, the chances for a positive outcome from the ethical evaluation increase. Furthermore, it was considered necessary that the checklist for costs, benefits and means must be rational and logical. In order to further develop ethical evaluation, a subsequent forum is planned to take place in Odense, Denmark in 2005.

Acknowledgements

The workshop was organised by the Cooperation Group for Laboratory Animal Sciences of the Finnish Ministry of Education and made possible through funding by the Academy of Finland, the Finnish Ministry of Education and Agriculture and Forestry, NOVA University and the Finnish Society for the Protection of Animals.

References

Bateson P (1986) When to experiment on animals. New Scientist **109***, 30-32*

COST	**BENEFIT**	**MEANS**
Pain	Human health	Experimental design - species, number - end points
Distress	Animal health	Alternatives
Discomfort and suffering	Safety (toxicity)	Facilities
Duration, frequency and severity	Increasing knowledge	Veterinary care
Death	Ecology	Training and competence
	Economy (macro)	Animal source and transport
		Negative results

Table 1. The factors included in the cost, benefit and means

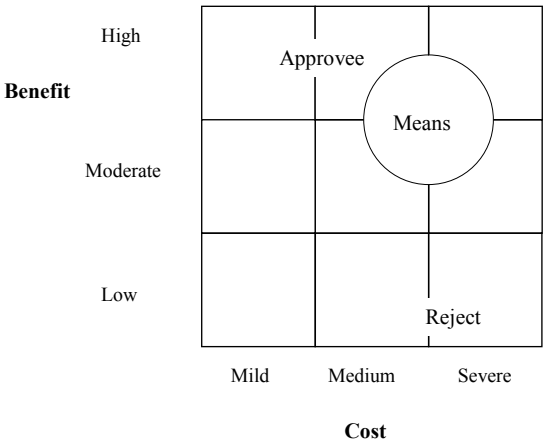

Figure 1. A model for cost-benefit analysis modified from that of Bateson (1986). The means has been added to the original model.

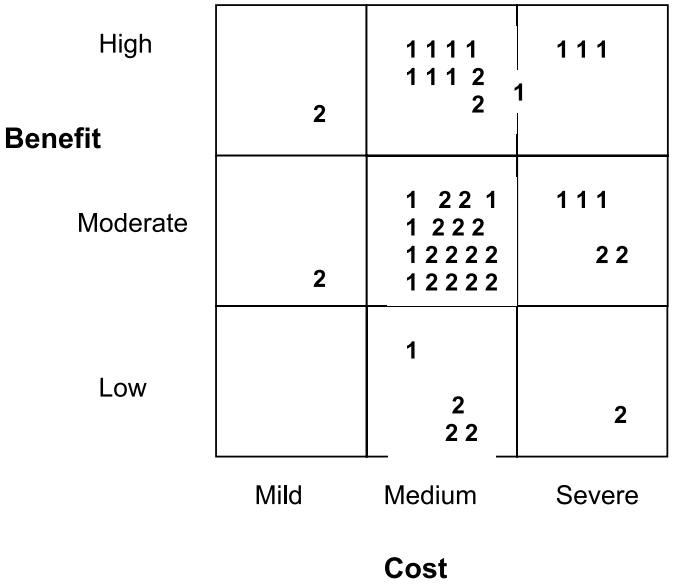

Figure 2. The results of the evaluation of the two case studies. The marks indicate the individual ethical evaluation scores. 1-marks show evaluation of the study 1 and 2-marks study 2.

Evaluation of experimental protocol applications in Greece based on EU regulations; is there a need for future revision?

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Summary

Applications for permission to conduct scientific research using laboratory animals in Greece are based on a questionnaire, according to the country's regulations, which have been harmonized to the European Union's Directive 86/609. The Prefecture Veterinary Service is responsible for evaluating the applications and granting or refusing permits. The issues that the authorities examine are the objective, the potential scientific benefit, the animals to be used, the method to be followed, the effect to their well-being and the research facility where the study will be carried out.

Throughout the time this procedure has been applied, observations have been made that certain improvements may be necessary, although they are not currently required by the country's regulations. In cases when insufficient description of the study's method is provided, the reviewers are prevented from estimating the extent of compromise to the animals' well-being. Revising the questionnaire with additional questions requiring extensive analysis could lead to a better-justified evaluation. Weaknesses observed in study design could also be prevented by a statistician's early input. The veterinarian's multiple roles in study design, experimental procedure and as establishment consultant could be enhanced in a revision of the legislation. Future amendments to the procedure currently in practice are proposed, with regard to the animals' well-being.

Introduction

The use of laboratory animals in experimental research is an issue that concerns lawmakers, inspectors and researchers, and is also a target of public criticism. The main point on which all parties agree is that laboratory animals have an intrinsic value and that man's respect towards them can be demonstrated by protecting their rights, *i.e.* ensuring their welfare or well-being.

Well-being is a complicated dynamic situation that can vary greatly among animals, as well as in the same animal from time to time (Clark *et al.* 1997). Because of the need to have a harmonized approach towards the enhancement of laboratory animals' well-being, several guidelines have been created. They assist the international scientific community for their optimal care and use, as well as to educate people involved with them on all levels (NRC 1996, FELASA Recommendations 1995, 1999, 2000). Legislation also ensures that humane care and treatment are provided to animals used in research facilities (European Directive 86/609/EEC, Dolan 2000). In Greece, experimental research using laboratory animals follows the country's regulations (P.D. 160/1991, Law 2015/1992), that have been harmonized to the European Directive 86/609/EEC regarding "the protection of animals used for experimental and other scientific purposes" since 1991. Emerging situations not anticipated or strictly controlled by legislation have shown there may be room for potential improvement regarding the protection of laboratory animal well-being.

The evaluation procedure in Greece

Veterinarians of the Prefecture Veterinary Service conduct the evaluation of the research protocol applications. They have to evaluate the cost to the animals' well-being versus the benefit to human or animal health, which will be accrued from the study, to warrant it. If alternative methods are justifiably not possible, the study's procedures regarding refinement and reduction are reviewed. Their recommendation to grant or refuse permission is proposed to the Prefect, who signs the permit.

The applicants are requested to fill out an eight-page questionnaire separated into 3 sections. The first section has questions regarding the study's design, the second about the animals to be used and the third about the research establishment. The questions have been selected so as to cover the articles of the Greek legislation (which is harmonized to the European Directive).

In the first section a description of the study is required. Discussions with the applicants have shown that, prior to their application, they have demonstrably carried out a thorough investigation of the references relevant to their study, and have the background knowledge necessary to support the answers regarding their study's objective, potential scientific benefit and method.

A percentage of the applicants give insufficient answers on the study's method. In 2003, this percentage in the Athens Prefecture applications was 25% (personal communication). This is mostly due to fear of disclosing information that could lead to the copying of the study and loss of its originality, even though there has never been a breach of confidentiality from the authorities. Insufficient information regarding the method prevents the reviewers from estimating the degree of discomfort or pain to be caused to the animals, not to mention further communications and delays. Providing details of the method could indicate whether adequate refinement measures have been considered to minimize discomfort. It is therefore suggested that a future modification of the original question on "description of the study" be the addition of sub-questions for a better welfare estimation.

In addition, the study's experimental design regarding the number of animals and experimental groups to be used must be fully described for the reviewers' evaluation regarding animal welfare, particularly the principle of reduction. It may be unrealistic to expect a researcher with a medical background to have training in experimental design and statistics. A biostatistician's input in the early stage of the study's design would minimize the number of animals to be used, consistent with achieving the desired scientific objectives. He could prevent common errors, which often result in the need to add more groups or more animals in the groups, or increase the duration, or even repeat the entire study, thus compromising refinement and reduction principles (Festing *et al.* 2002). He could advise on how to extract all

the useful information in the experimental data by appropriate statistical analysis and careful interpretation of results. Although neither the Greek law nor the Directive requires it, it is suggested that the addition of a biostatistician's opinion in the application will be a very valuable amendment and worth the inevitable increase in paperwork.

According to Greek law, each protocol application nominates a veterinarian, whose responsibility is to advise and ensure the procedures of the specific study are carried out in the animals' best interest. The applicants are required to discuss their protocol with the veterinarian, who most often is the person to help the applicants design the experiment. Being specialized in laboratory animal science, he/she can help them select the best animal model for their objective by informing them on species and strain differences, genetic and microbiological status, determine sample collection procedures, and select the most appropriate anesthesia and euthanasia techniques. The veterinarian is required to fill out a section in the questionnaire, stating his/her opinion on the study's prospective realization, regarding the degree of reduction to the animals' well-being compared to the potential benefit from the study's results. This cost – benefit assessment is of major importance for the reviewers' evaluation. A suggested improvement, which would ensure the veterinarian's assessment, could be that in the future this section be confidential, *i.e.* only for the reviewers.

The third part of the protocol application regards the research establishment in which the study will take place. According to Greek law, the facilities must be licensed and have all the necessary equipment. In their license, each research facility names a veterinarian as a permanent consultant. The research facilities however, are not required by law to employ the named veterinarian, full- or part-time. This lack of statutory appointment leaves both parties some relative freedom. It may result in insufficient or delayed advice on problems occurring throughout the day, during the veterinarian's absence. Even experienced technicians may not be able to handle some problems. A solution to this would be to amend the national legislation to require the employment of a veterinarian in the staff of a research facility. This would safeguard animal welfare considerably more.

A major revision in the evaluation process would also be to require the Prefecture Veterinary reviewers themselves to periodically obtain continuing training in principles of laboratory animal science and welfare. It is generally accepted that knowledge of new information regarding laboratory animal needs and optimal treatment, accompanied by sincere humane feelings towards them, is the best approach towards a responsible evaluation and licensing of research protocols.

Conclusions

The Greek evaluation process can be improved in several areas, not all of which have been discussed above. Minor changes and careful re-phrasing of the questionnaire currently in use can achieve some improvements. For other points, the national legislative body must be persuaded to amend the existing laws. Amendments that would strengthen the role of the veterinarian and would require the collaboration of a biostatistician will be beneficial to both laboratory animals and research. The study of procedures prevailing in other EU countries will certainly help find solutions. Finally, the protection of animal well-being in scientific research is an international issue that may be continuously improved by frequent revisions of the relevant legislation, as new knowledge accrues.

Acknowledgment

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Ethical review of outsourced protocols: addressing the co-responsibility of the sponsor and the contract research lab, a practical experience

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1. Introduction

Benefitting from long experience in the generation of induced or surgically modified rodents and the dedicated production of biospecimens and, more recently, an extension to experimental protocols, we designed a system covering our different activities, including breeding and "research services".

This system addresses not only the Protocol Review but also the organisation, training programme and key steps allowing a global "Ethical Review / Control" and to guarantee the implementation of the 3Rs concept for any project outsourced to Charles River Laboratories (CRL) France. The aim of this presentation was to initiate contacts and discussion with other groups, to learn from each other's experience.

2. Activities outsourced to CRL France.

The main activities include:

Research models, development and breeding.

these include surgically altered rodents, induced model generation (diet or treatment), transgenic and diagnostic services

biospecimens for *ex vivo / in vitro* use, breeding-related procedures including hysterectomy, embryo transfer, identification, blood sampling & biopsies.

Research and quality control services.

these include R&D protocols, drug and biological release testing.

Our Ethical Committee was created in 1991 but its activity, role and responsibility went through 3 major changes. The first was the extension of our service activities. The second, when its mission was extended to the 2 recently acquired French sites and their activities. The third followed the initiation of the corporate "Humane Care Policy"and the creation of a new corporate position to coordinate this programme.

In 2000, we joined the GRICE (French Ethical Committee Association) and in 2004, in order to better harmonize our activites a CRL European Ethical Group was set up, with a representative from each major European company.

3. Organisation of CRL Humane Care Policy and CRL France Ethical Committee.

The goal of this corporate programme is to assure that all CRL employees are committed to the humane care of the research animals produced and used in all CRL activities. The programme relies on several complementary approaches:

- To establish best practices across business units worldwide;
- To heighten internal awareness of the importance of humane care;
- To assure a culture of caring and openness;
- To enhance orientation and training;
- To develop processes to assure prompt recognition and correction of problems;
- To increase the worldwide recognition of CRL in the area of animal welfare and enrichment.

We divided the activities of our Ethical Committee in different categories:

- Implementation of the corporate Humane Care Policy;
- Protocol Reviews,
- Regular ethical audits of all activities and departments,
- Communication, training and awareness.

All these components are critical to achieve our objectives. The Ethical Committee is currently organized in 2 groups, with different responsabilities:

- A core group (currently the President of the Committee, a biologist and 2 veterinarians). It acts as an executive committee, in charge of protocol reviews (entire core group) and ethical audits (at least by 2 members, at least one being a vet). In the near future, we plan to add one member who is not involved in animal use
- The full committee, with the core group plus one officially appointed representative for each activity or department. It meets at least twice a year and acts as a board of management, to define the objectives and to monitor the work of the core group.

At least once a year, the full committee meets for an annual review. Representatives of a National Animal Welfare Association, our local veterinary inspectorate and one customer representative are also invited.

4. Ethical Audits.

The goal of ethical audits is to review as critically as possible all standard procedures and practices related to the maintenance and use of animals. Working in close collaboration with the operational teams makes it possible to identify a wide range of improvements in the field of animal care and welfare, housing and caging, technical refinements, education and training.

5. Education and Training.

With the development of a culture of personal awareness and responsibility, education and training are the cornerstone of our Humane Care Policy. Activities include:

- An introduction to our "Humane Care Policy"

- Regulatory programmes (FELASA categories A, B and C);
- Practical and technical training (from basic handling, identification, administration and blood collection procedures);
- Pain & distress recognition and management…

6. Protocol Reviews, implementation of 3Rs and split of responsibility.

Before outsourcing an *in vivo* study, the Contract Research Organisation (CRO) and the Sponsor have to guarantee the strict implementation of the 3 Rs principles.

In some fields (such as non regulatory studies or assays) the sponsor is the only one to control all aspects of the study and the number of animals used. However, it is the responsibility of the CRO to check that the Sponsor before outsourcing has conducted a Protocol Review.

With sensitive studies, additional measures to guarantee the quality of the "justification" and the "replacement" steps may be required before acceptation of the project.

In some cases, due to a lack of justification, poor documentation or a refusal to accept major ethical improvement(s), we had no other option than to reject the protocol submitted.

Later both the sponsor and the CRO can collaborate very closely on further reduction issues and on all aspects of refinement. The most frequent improvements to the protocols received are:

- Pain evaluation;
- Use of end-points (tumours, infections);
- Blood sampling: route / technique, volume, frequency;
- Administration volumes & doses;
- Surgical technique;
- Pain evaluation and treatment;
- Anaesthesia, analgesia, and post-operative animal care;
- Euthanasia;
- Husbandry, shipment conditions;
- Fasting duration;
- Osmotic pumps, telemetry, chronic cannulation.

For end points, we mainly but not exclusively refer to the "Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals used in Safety Evaluation" (OECD 2000).

In other fields, such as surgically modified rodents or transgenic services, the Protocol Reviews and ethical audits made it possible to bring major improvements to such things as analgesia, anaesthesia, identification techniques, catheters and telemetry implantation, blood sampling, euthanasia, shipping crates, post-procedural care, customer instructions, deviation reporting, corrective actions, and quality assurance.

In accordance with our corporate Humane Care Policy, it is our intention to keep improving our practices and "raising the bar" in the field of animal care and welfare.

The refining influence of Ethics Committees on animal experimentation in Sweden

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Ethical review processes are being introduced in a number of countries. An important aim is to stimulate the introduction of the Three Rs. Mandatory scrutiny of projects by animal ethics committees was introduced in Sweden in 1979 and the present study was conducted with the aim to assess whether the ethical review process had had a refining influence on animal experimentation in this country. We (JoH) investigated the minutes of meetings held between 1989 and 2000 at which consideration of applications for experimental work in animals resulted in requests for modification (n = 3607). 18.1% of the applications received were approved only after modifications. The majority of the changes requested may be classified as 'Refinement'. The most common requests were for improvement of project design, euthanasia method and housing and husbandry. There was a relative increase in modifications requested by the committees related to anaesthesia, choice of licensed supervisor and the need for licenses or informed consent from animal owners during the period investigated. There was a relative decrease in modifications related to euthanasia, housing and husbandry, and general endpoint assertions. The results suggest that the work of the committees may be perceived as an ongoing process, since several of the applications for which modification was requested were projects that had been approved on a previous occasion but were now up for renewal. In order to have maximal influence on the refinement of scientific protocols it is important that the scientists in the committees are continuously updated on developments in laboratory animal science.

Development of a welfare-benchmarking scheme for laboratory mice

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Summary

A welfare-benchmarking scheme has been developed for laboratory mice, which allows establishments to compare the results of their own welfare assessment with those of their peer group with the aim of improving welfare standards through education and encouragement. The key elements are an assessment protocol, profile of the peer group results, guidance notes and training. The welfare assessment protocol was developed to evaluate the impact of husbandry and housing on laboratory mouse welfare through expert consultation, comprehensive testing and refinement to ensure that all measures were valid, reliable and feasible. This protocol was used to assess the welfare of laboratory mice at 46 UK animal units using an establishment questionnaire and observations carried out during a one-day visit. The data was entered into a rolling database of welfare performance that presented the results with an anonymous summary of the findings nationally, allowing establishments to identify welfare strengths and weaknesses. Guidance notes and training programme have been developed to assist with the assessment protocol, results database and benchmarking process. Further close consultation with the laboratory animal industry will be necessary, as implementation of a benchmarking scheme depends on the willingness of industry to use it.

Introduction

Welfare benchmarking refers to the process by which individuals are able to compare the results of their own assessment with those of their peers. This has proved to be a powerful motivational tool for improving and maintaining high standards of farm animal welfare (Whay, et al., 2003), as it "… prevents participants burying their head in the sand or accepting a certain level of disease [or poor welfare] as normal" (Huxley et al., 2003). The key elements of a benchmarking scheme are an effective assessment protocol, profile of the national group results, and guidance materials. This allows individuals to measure and review their own welfare performance, so that they can modify existing procedures to improve welfare.

The objective of the welfare assessment protocol is to evaluate the impact welfare has on the way in which we keep animals. This concept has not been widely applied to laboratory animals although the precedent for assessing aspects of health and welfare already exists in the post-operative and post-procedure monitoring of pain, distress and discomfort (Per Obs., 2003; Hawkins, 2002). However, these systems have not been extended to assess the effect of housing and husbandry on laboratory animal welfare.

Until recently the development of welfare assessment schemes have predominately focused on farm animal species (Sørenson & Sandøe, 2001; Webster & Main, 2003), although, the principles are applicable to laboratory animal welfare. To assess welfare, the resources provided by an establishment and its staff to the animals in their care has to be measured (resource inputs) together with the behavioural, physiological and pathological reactions of animals to what their life experience (animal-based outcomes). The assessment of both resource inputs (e.g. housing, husbandry, diet, the environment, management policies, and stockmanship) and animal-based outcomes (e.g. behaviour, health, physical appearance, and breeding performance) is crucial for a comprehensive and holistic assessment of animal welfare.

The measures that comprise any welfare assessment protocol must also be valid for assessing welfare, within the constraints of the housing conditions and reliable over time, and between assessors and establishments (adapted from Winckler, et al., 2003). Finally, welfare assessment has predominately focused on evaluation of individuals as welfare is often defined in this way (Brambell, 1965; Hughes, 1976; Hurnik et al., 1985; Barnett & Hemsworth, 1990; Duncan, 1993; Fraser et al., 1997). Despite this, we believe that the welfare of individuals can be effectively assessed at the group or unit level as animals within a unit share many common experiences that will affect their welfare such as their environment, husbandry and veterinary care, staff attitude, and institutional policies/investments.

The profile of peer group results with an anonymous summary of the findings nationally enables each establishment to compare their performance with that of their peers. To effectively measure and benchmark their own welfare performance, establishments require appropriate guidance notes and training to include the use of the protocol, the results profile, and interpretation of the results to identify their significance.

The development of a welfare-benchmarking scheme for laboratory mice addresses some the recent recommendations/ requirements put forward by various regulatory and advisory bodies in the UK concerning development of a method to assess welfare. The Home Office in a recent review of the Local Ethical Review Process (Home Office, 2001), stated that it required a objective welfare assessment scheme to fulfil one of its main roles, to ensure that the 'Best standards of care and accommodation are sought and implemented for laboratory animals'. The House of Lords Select Committee on Animals in Scientific Procedures (House of Lords, 2002), stated the need for a welfare assessment system that could be carried out by named veterinary surgeons, named animal care & welfare officers, & animal technicians (Recommendation 31). Finally, in response to the Select Committee's report, the UK Government (2003) stated the need for a framework that could gather information on the 'life experience' (Paragraph 53). The assessment protocol that forms an integral component of a welfare-benchmarking scheme could fulfil these needs. As it offers a valid assessment method that provides the necessary supporting structure and is carried out by those who are responsible for the care and use of laboratory animals.

Objectives

The aim of this project was to develop and implement a benchmarking scheme to improve and maintain high standards of laboratory mouse welfare in UK animal units, which was achieved by completing the following objectives:

· Development of an expert defined welfare assessment protocol for laboratory mice that evaluated welfare and used measures that were animal-based, minimally disturbing and non-invasive.

· Implementation of the protocol scheme to assess the welfare of conventional (genetically normal) laboratory mice that were not under procedure.

· Development of a database of national results that would profile mouse welfare in UK animal units.

· Development of a scheme that could be used by all of those responsible for the care and use of laboratory mice within an establishment.

Consultation process

The initial stage involved the identification of valid measures of mouse welfare. This was accomplished using the Delphi consultation process, a 2-stage technique designed to gather expert opinion and achieve some degree of consensus (Linstone & Turoff 1975). This process (Leach, et al., 2004) will be summarised. In the first stage a variety of experts were approached and asked to identify potential measures of mouse welfare and appropriate sample sizes and sampling times for those measures. In the second stage the results of the first stage were return to the experts and they were asked rank and comment on the measures, sample sizes and sampling times that had been selected. A considerable degree of consensus was reached at the end of the second consultation stage with a total of 97 measures being identified as appropriate for assessing mouse welfare. Of these, 55 were resource input measures and 42 were animal-based outcome measures (see un-shaded measures in Table 1). These can be separated into 12 categories according to what they measure; cage specifications, environmental conditions, husbandry procedures, provision of food and water, details about staffing, presence and use of resources in mouse cages, unprovoked behaviour, provoked responses, vocalisations, physical appearance, presence of people and other species in mouse rooms, individual health, and health issues.

Formulation of assessment protocol

The next stage of the developmental process was to formulate a valid and reliable protocol based on the 97 expert-defined measures identified in the consultation stage. The measures were separated into those that could be recorded by observation during a one day visit (max 8 hours) to each unit and those could be recorded using a questionnaire completed by the staff prior to the visit (see Table 1).

At this stage all measures were considered to have achieved a degree of validity. The reliability of the measures recorded by observation were then tested at four animal units in the UK. Feasibility was assessed in terms the practicality of each measure for assessing welfare within the constraints of an animal unit and this project.

The first constraint was that samples could not be taken from inside of the mouse cage because of risks to containment and disturbance. The second was that any equipment for collecting data must be portable, available, cost-effective, capable withstanding sterilisation, and not pose a biosecurity hazard. Finally, the reliability of those measures considered to be feasible was assessed during the national assessment of mouse welfare to ensure that they were reliable both between assessors and over time. The majority of measures were found to have good reliability, although some measures exhibited considerable variability.

Changes to the protocol

With testing and refinement, 15 of the 97 measures ere removed from the protocol (see strikethrough measures in Table 1). Ultrasound levels were excluded because the equipment needed was costly and unsuitable for use in an animal unit. Concentrations of ammonia, particulates, and carbon dioxide and the response to a novel object were excluded because part of sampling device or the novel object has to be placed into the mouse cage. Staff attitude was excluded as it was not considered feasible within a one day visit and many of the establishments were unwilling for their staff to be interviewed. Response to auditory stimulus and respiration rate were excluded because it was difficult to assess without causing considerable disturbance to the animals. Audible vocalisation was excluded because it was difficult to differentiate it from any background noiset. Faecal glucocorticoids and faeces/urine output were excluded because sampling involved opening the cage to remove samples. Cage cleanliness was excluded because it date dependant. Finally, presence of other mouse cages and cage safety were excluded because they were assessed by other measures.

In addition, 27 new measures were added to the protocol (see shaded measures in Table 1), which were related to those identified in consultation stage and included 6 resource input and 33 animal-based outcome measures.

Assessment of mouse welfare in UK animal units

The refined protocol contained a total of 68 resource inputs and 51 animal-based outcome measures (see Table 1). These were separated into 13 categories and these measures were allocated to an establishment questionnaire or as observations made during the one day visit.

The resource questionnaire was sent approximately 2 weeks before the scheduled visit, and contained questions concerning almost all of the resource input measures and some of the animal-based outcome measures. The one day visit included recording of all the animal-based outcome measures, the resource-based input measures that were not covered in the questionnaire, and a number of resource input measures included in the establishment questionnaire to ascertain whether there was a difference between reported (questionnaire data) and observed levels (observation data). This protocol was then used to assess the welfare of laboratory mice in 46 UK animal units between April and December 2003. This involved visiting a total of 22 commercial, academic and research establishments that ranged in the number of mice they housed from a few hundred to the many thousands. An animal unit was defined for the purposes of this project as all of those mice cared for by one Named Animal Care Welfare Officer. Potential establishments were approached by the authors and given a detailed description of the project and then asked to participate in this study. Of those establishments approached only four choose not to participate due to bio-security concerns.

Profile of national results

The data collected during the national assessment was entered into a rolling web-based database of welfare performance. This contained no reference to the identities of the participating establishments or individuals but each institution was able to gain secure access to the database. Unit and national results gave establishments the ability to compare their own welfare performance and with that nationally, so enabling them to identify resource strengths and weaknesses. It is hoped that establishments will continue to use the assessment protocol and update this database periodically so that it could become a source of information for the future.

Guidance notes and training

Comprehensive guidance notes and training materials have been developed to enable establishments and their staff to use this scheme. These offer a guide to the use of the database as well as interpretation of the results. They provide a detailed definition and description of the methodology used and an explanation of its significance. Training would also include discussion of the significance on each measure and how the database works. The aim of the guidance notes and training was to ensure consistency between assessors and establishments. It also enabled establishments to interpret their results, determine their welfare performance and identify their strengths and weaknesses.

Benchmarking scheme

This scheme offers establishments a management tool to measure and benchmark their own welfare performance. It aims to provide staff with a simple system of recording and reviewing performance, enabling them to modify existing procedures. We hope that this will encourage all staff in improving and maintaining high standards of welfare within their establishment. In accordance with the saying, "measure what you treasure, and manage what you measure" welfare assessments performed by staff within institutions are an essential component of welfare management. We believe that educating and encouraging staff is more likely to lead to improvements in welfare standards than enforcement through legislation and codes of practice alone, and is a more positive way of achieving the goal of high welfare standards.

This welfare scheme could also provide an objective method of assessment for enforcement agencies who are responsible for evaluating compliance with animal welfare related standards or legislation.

Further implementation

Despite the recommendations put forward by regulatory bodies, such as the Home Office (Home Office, 2001), the House of Lords Select Committee on Animals in Scientific Procedures (House of Lords, 2002) and the UK Government (UK Government, 2003), successful implementation of any system will be depend on the industry itself being willing to use it. This in turn will depend on the industry being encouraged to use a system that they perceive as valid, reliable and above all feasible within the constraints of commercial animal facility.

The first step to successfully implement such a scheme will be to ensure that it comprises only measures that are the most valid, reliable and feasible for assessing welfare. Further assessments of validity will be accomplished by using the data collected during the national assessment and through close consultation with the industry itself.

The second step will be to encourage the industry to implement such a scheme with support from animal welfare charities, regulatory authorities, and funding bodies. Secondly, enforcement by the regulatory authorities making the implementation such a system a requirement of those establishments using animals. This could also apply to the funding bodies, which could make implementation of a system a requirement of the funding that they offer. Finally, through education of all those responsible for the care and use of experimental animals, so that the benefits of a benchmarking system can be fully appreciated for both the animals and the validity of the resulting research. The various professional groups, such as IAT, LASA, and LAVA, could undertake this. We believe that a combination of these three approaches will be necessary and the most effective at ensuring that a necessary welfare-benchmarking system is adequately implemented.

Future development

For any scheme to continually improve and then maintain welfare standards it must incorporate a dynamic assessment protocol that can be adapted to local circumstances and modified as improved welfare measures become available so that the most valid and reliable measures are always used. An adaptable protocol, guidance notes, training and a profile of national results would allow this scheme to be adapted for genetically modified mice, those undergoing procedures and potentially other laboratory animal species.

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Table 1 The measures and categories used to assess mouse welfare divided into resource-based and animal-based outcome measures and indicating whether they were recorded via observation or establishment questionnaire. The un-shaded measures refer to the those identified through expert consultation (see Leach et al., 2004). The measures with a strikethrough (Cage safety) refer to those removed and shaded measures refer to those added after testing and refinement.

Measure		Resource-based inputs	Animal-based outcomes	Observation	Questionnaire
Cage specifications:					
	Stocking density		✓	✓	✓
	Group size		✓	✓	✓
	Cage dimensions	✓		✓	
	Cage opaqueness	✓		✓	✓
	Cage material	✓		✓	
	Floor type	✓		✓	✓
	Cage gnawing		✓	✓	✓
	~~Cage safety~~	~~✓~~		~~✓~~	
	Proximity		✓	✓	
Cage environment:					
	Biosecurity	✓		✓	✓
	Temperature	✓		✓	✓
	Humidity	✓		✓	✓
	Room light intensity	✓		✓	✓
	Covered cages	✓		✓	
	Cage light intensity	✓		✓	
	Photoperiod times	✓			✓
	Light source	✓		✓	✓
	Audible noise level	✓		✓	✓
	Ventilation type	✓		✓	✓
	Background music	✓		✓	✓
	~~Ultrasonic noise level~~	~~✓~~		~~✓~~	
	~~Ammonia levels~~	~~✓~~		~~✓~~	
	~~Particulate levels~~	~~✓~~		~~✓~~	
	~~CO_2 levels~~	~~✓~~		~~✓~~	
Husbandry:					
	Cage cleaning method	✓			✓
	Cage cleaning frequency	✓			✓
	~~Cage cleanliness~~	~~✓~~		~~✓~~	
	Handling competence	✓			✓
	Within institution transport	✓			✓
	Identification method	✓		✓	✓
	Weaning age	✓			✓
	Interference during sleep phase	✓			✓
	Regrouping animals after initial grouping	✓			✓

	Euthanasia method	✓		✓
	Euthanasia with conspecifics present	✓		✓
	Quality of facilities for sick/injured	✓		✓
	Euthanasia of sick/injured animals	✓		✓
	Lights on during the dark period	✓		✓
Provision of food/water:				
	Food type	✓	✓	✓
	Presence of floor food in cage	✓	✓	✓
	Presence of floor food in animal room	✓	✓	
	Watering method	✓	✓	✓
	Occurrence of flooding	✓		✓
Staffing details:				
	~~Staff attitude~~	~~✓~~		~~✓~~
	Staffing levels	✓		✓
	Inspection of animals	✓		✓
	Ease of observation	✓		✓
	Staff training	✓		✓
	Accreditation scheme	✓		✓
	Availability of welfare information	✓		✓
	Availability of records	✓	✓	✓
Presence of resources in mouse cages:				
	Substrate type	✓	✓	✓
	Nesting material type	✓	✓	✓
	Shelter type	✓	✓	✓
	Shelter transparency	✓	✓	
	Gnawing material type	✓	✓	✓
	Visual barrier	✓	✓	✓
	Wheel type	✓	✓	✓
	Other forms of enrichment	✓	✓	✓
Use of resources in mouse cages:				
	Nesting material	✓	✓	
	Shelter	✓	✓	
	Gnawing material	✓	✓	
	Wheel	✓	✓	

Unprovoked behaviour:				
	Behavioural repertoire	✓		✓
	Positive active behaviour	✓	✓	
	Negative active behaviour	✓	✓	✓
	Abnormal behaviour	✓	✓	✓
	Type of abnormal behaviour	✓	✓	
	Inactivity	✓	✓	
	Climbing	✓	✓	
	Digging	✓	✓	
	Out of sight	✓	✓	
	Gnawing	✓	✓	
	Wheel use	✓	✓	
	Positive parental behaviour	✓	✓	
	Negative parental behaviour	✓	✓	
	Other behaviours	✓	✓	
Provoked responses:				
	Freeze duration	✓	✓	
	Hide duration	✓	✓	
	Inquisitive mice	✓	✓	
	~~Novel object response~~	~~✓~~	~~✓~~	
	~~Auditory response~~	~~✓~~	~~✓~~	
Vocalisations:				
	~~Ultrasonic vocalisations~~	~~✓~~	~~✓~~	
	~~Audible vocalisations~~	~~✓~~	~~✓~~	
Physical appearance:				
	Alertness	✓	✓	
	Posture	✓	✓	✓
	Wall hugging	✓	✓	✓
	Barbering	✓	✓	✓
	Physical damage	✓	✓	✓
	Starey coat	✓	✓	✓
		✓	✓	✓
	Body score	✓	✓	
	Skin colour	✓	✓	
	Ocular/nasal discharge	✓	✓	✓
	Openness of eyes	✓	✓	
	Sunken abdomen	✓	✓	
	Hair loss	✓	✓	✓
	Pinched face	✓	✓	
	Gait	✓	✓	
	Tail position	✓	✓	
Presence of others in mouse room:				
	Room activity	✓	✓	✓
	~~Presence of other mouse cages~~	~~✓~~	~~✓~~	
	Presence of other species	✓	✓	✓
Individual health:				
	Weight changes	✓		✓
	Obvious signs of disease	✓	✓	
	~~Respiration rate~~	~~✓~~	~~✓~~	
	Respiration type	✓	✓	

	Blood/Salvia in bedding		✓	✓	
	Nervous problems		✓	✓	
	~~Faeces and urine output~~		~~✓~~	~~✓~~	
	~~Faecal glucocorticiod level~~		~~✓~~	~~✓~~	
Unit health:					
	Disease incidence	✓			✓
	Health	✓			✓
	Mortality	✓			✓
	Welfare included in ethical review	✓			✓
	Health screening	✓			✓
	Disease limitation methods	✓			✓
	Hazard assessment	✓			✓
Response to capture/handling/restraint:					
	Capture time		✓	✓	
	Pick up method	✓		✓	
	Supported	✓		✓	
	Placement method	✓		✓	
	Handling type	✓		✓	
	Handling speed	✓		✓	
	Capture score	✓		✓	
	Aggression during capture		✓	✓	
	Vocalisation during capture		✓	✓	
	Restraint type	✓		✓	
	Restraint method	✓		✓	
	Restraint time	✓		✓	
	Struggling against restraint		✓	✓	
	Biting during restraint		✓	✓	
	Vocalisation during restraint		✓	✓	
	Restraint score	✓		✓	

Comparative Functional Genomics : Animals get closer to Human

Mouse Genome Project: the repercussions of sequencing on the analysis of phenotypes

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Since the last FELASA meeting, the mouse and rat genomes have been entirely sequenced. These sequencing efforts have permitted interspecies comparisons with the human genome resulting in the identification of conserved sequences with functionality, indicating that about half the genes in these species are not yet known. These sequencing efforts have also provided a wealth of data that will have a major impact on the management of mouse and rat colonies and, in a more general way, on the design of experiments making use of these two species. When the genome sequence from a given inbred strain (for example C57BL/6) is aligned with sample short sequences from other strains, one observes a variety of single nucleotide polymorphisms, or SNPs. These SNPs are not evenly distributed; on the contrary, they are mostly in the intronic regions and much less frequently in the exons. Their density is also extremely variable with segments often extending across tens of megabases of extremely high (about 40 SNPs per 10 kb) or extremely low (about 0.5 SNPs per 10 kb) polymorphism rates depending on the strains and the region considered. The junctions between any two segments are delineated by abrupt transitions defining haplotypes indicating a mosaicism or heterogeneity at the genome level. In all strain-to-strain comparisons examined, about one-third of the genome falls into long regions of a high SNP rate, consistent with estimated divergence rates between *Mus musculus domesticus* and either *M. m. musculus* or *M. m. castaneus*. These data confirms the former observations that the genomes of inbred strains are mosaics with the vast majority of segments derived from either *Mus m. domesticus* or *Mus m. musculus* sources. Making a catalogue of SNPs across the major laboratory inbred strains can be used to identify 'strain specific regions' , which in turn would allow to design very rigorous tests for genetic monitoring. Using these tests it should be possible to identify the origin of a potential contamination, something that is difficult or impossible with the classical assays. These observations also have implications for the design and interpretation of positional cloning experiments. They will allow for example to identify the origin of the chromosome in which a spontaneous mutation occurred when the latter is unknown. They will also allow setting the best and most informative cross to reduce the critical interval. The differences of polymorphisms between genomes of different strains are also of great interest because they can be associated with or even underlie phenotypic traits, including disease susceptibility. This is why a catalogue of SNPs will undoubtedly prove valuable for those seeking to map mutant phenotypes and elusive QTLs in the genome. This paper is related to a paper by Doctor Molly Bogue about the Mouse Phenome project.

The Mouse Phenome Project: understanding human biology through mouse genetics and genomics

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Mice have been used for decades to study human physiology and disease. The remarkable similarity of mouse and human genomes, in both synteny and sequence, validates the mouse as an exceptional model organism. With the availability of high-accuracy sequence of the mouse genome and haplotype information for over 40 inbred strains, a new and powerful paradigm for biomedical research is established. Haplotype maps of inbred mouse strains combined with sophisticated delineation of their phenotypic variation and gene expression patterns will enable genetic analyses on an unprecedented scale. Inbred mouse strains provide a genetically stable and genetically defined tool for research. As reproducible entities of uniform physiology and genetics, inbred strains can be studied over time and in many locations worldwide. Data generated are cumulative and valuable to the research community. The Mouse Phenome Project is an ongoing international collaborative effort 'trial participation' typic characterization of a defined set of mouse strains under standardized conditions and to make the data publicly available through a web-accessible database. Data for a wide range of parameters are annotated and stored in the Mouse Phenome Database (MPD) - along with submitter's contact information, detailed protocols, and environmental parameters. Genotypic data are collected in parallel. Tools for data retrieval and analysis are available through a website interface. Universal access to centralized strain data enables investigators to choose appropriate strains for modelling disease processes, physiological studies, toxicology, disease susceptibility research, and other systems-based approaches. The Mouse Phenome Project maximizes community resources by collecting universally useful biological data while minimizing the number of animals needed for research.

The European Mouse Mutant Archive

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Summary

The European Mouse Mutant Archive (EMMA) is a non-profit repository in which medically relevant mouse mutant strains, essential for basic biomedical research, can be preserved. Qualified research scientists can readily access these strains for the purpose of academic research. In order to make the transfer of biological information efficient, appropriate databases have been established in which all the genetic and phenotypic properties of the mutant strains that EMMA stocks are described. EMMA is supported by the European Commission under Framework Programmes 5 and 6, and by the participating institutions.

Introduction

The mouse is currently the best model to investigate the biological functions of genes and inherited diseases in humans. In order to meet the requirements of functional human genome analysis, a large number of different mouse mutants are needed. It is also essential that all the mutants be maintained in well-organised repositories, where they can be readily available to researchers. To meet these requirements, EMMA was established and implemented as a state-of-the-art mouse mutant repository at the service of the international scientific community.

By offering mouse sperm or embryo cryo-preservation as an economical alternative to maintaining genetically unique strains of mice, EMMA answers many major problems that the scientific community had previously been faced with such as high costs of maintaining valuable strains as live mice, insurance against the loss of a strain due to disease or genetic changes. Its main objectives, therefore, are to stock, preserve, and redistribute the mouse mutant strains that scientists produce. EMMA thus plays the role of a mediator between the depositor and the scientist interested in working with specific mouse strains, in a process in which existing Material Transfer Agreements retain their validity.

Partners

Currently, EMMA is run by seven partner organisations from six European countries. Every partner is a major player in the field of mouse genetics. The Italian partner is the Consiglio Nazionale delle Ricerche, Instituto di Biologia Cellulare (CNR-IBC) at Monterotondo (Rome), which materially manages the main EMMA repository, archiving mouse mutant strains mainly in the form of cryo-preserved embryos. The EMMA database and web server are located at CNR-IBC, and courses on cryo-preservation techniques are held there every year. The French partner (the Centre National de la Récherche Scientifique, Centre de Distribution, de Typage et Archivage Animal (CNRS.CDTA) in Orleans), the British partner (the Medical Research Council, Mammalian Genetics Unit (MRC.MGU) in Harwell), and the GSF Research Centre for Environmental and Health, Institute of Experimental Genetics (HGF.GSF) in Neuherberg, all have long-term expertise in cryo-preservation and re-derivation of mouse mutant lines. In addition to providing archival services, the Swedish partner (the Karolinska Institute, Clinical Research Centre, Unit for Embryology and Genetics (KI.MEG) in Stockholm) and the Portuguese partner (the Fondacao C.Gulbenkian, Instituto Gulbenkian de Ciencia (FCG.IGC) near Lisbon) have also established germ-free facilities and are able to provide mice of germ-free status. The remaining partner in the consortium, the European Bio-informatics Institute (EMBL-EBI) in Hinxton, provides bio-informatics and support expertise, and is responsible for the databases.

Organisation

The consortium has afforded huge potential to EMMA as the central mouse mutant repository in Europe, and as part of a worldwide network of repositories. To guarantee trouble-free procedures, different organisational levels have been implemented. An International Project Policy Committee (IPPC) consisting of experts in the field of modern mammalian genetics ensures that the repository operates at appropriate standards of quality. The Technical Working

Group (TWG) is composed of the leading hands-on scientists from every partner organisation. The committee discusses and agrees on EMMA Standard Operation Protocols (SOPs) for quality control, archiving, and distribution. It has also created a platform to discuss scientific problems, trends in archiving, animal husbandry, and the distribution of living animals and frozen germ plasm.

Prof. Martin Hrab de Angelis, the director of the Institute of Experimental Genetics at the GSF-National Research Centre for Environmental and Health, is EMMA's scientific director and the person responsible for general co-ordination. He heads the Board of Participating Directors (BPD), which discusses the recommendations of the other Boards, and represents the top decision-making level within the EMMA consortium. All these various groups meet periodically to make decisions about EMMA and to ensure that it is functioning at the appropriate, high standards of quality.

Procedures

When using the EMMA on-line submission form, the depositor is asked to provide all relevant information on his mouse mutant line. These data are then transferred to the EMMA database and form the basis of the information that will appear on the EMMA website. The information that is provided, therefore, must be entirely reliable. The depositor is asked to provide information on the mutant, such as kind of mutation, defect, genetic background, breeding history, references, the existence of Intellectual Property Rights, etc. The applications are forwarded to the EMMA Scientific Review Committee for evaluation. The Evaluation Committee is made up of four experts from the field of modern mouse genetics—scientists with a great amount of expertise in different areas of the field of mouse genetics and in the production of genetically-manipulated mice—and ensures the quality and value of the mouse mutant strains that are made available. The Committee looks for clearly-described phenotypes with obvious evidence of heritability and an identifiable genotype; by evaluating the potential importance of specific mouse lines for current and future research, it ensures that the scientific community will be able to access mouse mutant strains of real scientific value.

After a strain is approved, mice of breeding age with a health-status report not older than three months are sent to one of the EMMA facilities for embryo or sperm cryo-preservation. In which facility the mouse will finally be archived mainly depends on where the mouse comes from and what its genetic background is. EMMA tries to keep the number of shipments of specimens, and therefore also the costs of transferring them, at a minimum. Genetic background is, after other logistical considerations, the most important criteria used to decide in which form, whether as sperm or as embryos, the mouse mutant strain will be archived. To ensure a high level of quality, various control procedures have been created. The animals are monitored genetically and phenotypically. Various SOPs have been developed for freezing procedures, health status, animal handling, and the transfer of frozen or living mice, all of which help to maintain the quality level high. To ascertain the ability to reconstitute a stock from frozen, embryos produced from frozen sperm are thawed, transferred into pseudo-pregnant recipients, and recovered as live-born mice which are then reared. It is possible to guarantee the highest health standards since health monitoring is performed on all incoming and outgoing mouse lines. Every mouse line within the EMMA programme undergoes intensive health monitoring. All mice must obtain a specific-pathogen-free (SPF) status, according to FELASA rules, which is certificated. The importation programme includes the immediate isolation of mice that are delivered into flexible film isolators and IVC-racks, health screening, and embryo derivation of an F1 strain generation in SPF-barrier maintained foster mothers.

Afterwards, all foster mothers and selected progeny are sampled for complete parasitology, bacteriology, and virology by ELISA, IFA, and PCR assays according to the FELASA recommendations. Health analyses are constantly reviewed and updated in order to raise the sensitivity of pathogen detection. In addition, EMMA has established effective strategies to assess the health status of barrier-maintained colonies, such as systematic sentinel sanitary monitoring, controlled decontamination of equipment and supplies that enter facility, proper SPF procedural reviewing, biological contaminated material wasting, appropriate husbandry procedures, etc.

When the diagnosis is confirmed, appropriate procedures are adopted to control the pathogen risk, such as depopulation, bio-containment, chemotherapy and, when necessary, embryo-derivation. To maintain these quality standards, a systematic effort is necessary to evaluate, prevent, and sort out adverse infections. Moreover, within the standard workflow of the cryo-preservation procedure, the different EMMA partners continuously exchange frozen probes for re-derivation to ensure that the protocols work successfully. In general, 500 embryos or 50 sperm samples per line are archived.

EMMA-maintained lines are supplied to qualified investigators as a service solely for research purposes and not for commercial reasons. It is also laid down that the recipient individual, laboratory, or institution may not transfer or sell the mice or their progeny to any third party outside the recipient institution.

Up to three breeding pairs can be provided on request with little or no delay, while cryo-preserved strains can be sent as frozen samples in (8 cell stage) two straws containing 20-25 embryos, alternatively 3 straws with sperm) or re-derived upon request. All animals provided by EMMA thus obtain SPF status according to FELASA rules. The depositor has to pay the transportation costs for the mouse from his or her facility to the archiving EMMA centre. The requestor also pays the transportation cost from the EMMA centre to his or her facility and, in addition, a fee of 200 Euro, which does not vary whether the mice supplied are frozen or alive.

Special services

EMMA has already established a service for germ–free breeding and has an increasing number of available genetically-modified mouse strains.

As a special service, EMMA provides the pertinent nomenclature for the archived mice. EMMA works closely together with the Mouse Genomic Nomenclature Committee (MGNC), which follows the rules and guidelines established by the International Committee on Standardised Genetic Nomenclature for Mice. The data on the mice are collected and sent to the MGI for approval. The standardised nomenclature is especially important because all the mutant mouse repositories in the world will build-up a virtual, central archive.

Advantages

Any producer of a mouse mutant line who archives mice

in EMMA can be sure of a safe and clean back-up of the line. The mice are archived and maintained under the highest standards. The producer will increase his or her number of citations without relinquishing any intellectual property rights. All mice supplied by EMMA obtain SPF status according to FELASA rules.

Future

Given that the number of mouse mutant strains being produced, including knock-outs, knock-ins, gene-trap mice and transgenic mice is continuously increasing, it is essential that all mutants be retained and kept in a well-organised, central repository from which they may be made available. Mutants can represent a step in the development of targeted drugs and help to improve our understanding of the molecular basis both of diseases and of normal development.

In the near future, EMMA will become the European part of the worldwide network of repositories. The data on mice stored in EMMA will become part of the international mouse databases such as the International Mouse Strain Resource (IMSR). The IMSR provides a catalogue on the World Wide Web of all available laboratory mouse stocks and mutations extant worldwide. Moreover, the strong co-operation between EMMA and the JAX (The Jackson Laboratory) in Bar Harbour, Maine, USA, which serves as the principal repository for mutant mice in the United States, will be continued. EMMA also continuously exchanges experiences with the Mouse Mutant Regional Resource Centre (MMRRC). The MMRRC represents a group of academic and commercial facilities for storing and distributing mutants that began operating in 2001 and currently fulfils in the U.S.A. the same function that EMMA has in Europe.

It has been essential to make a concerted effort to archive and distribute the most valuable lines within Europe. EMMA has cryo-preserved hundreds of strains, and the rate of request for these strains has been constantly increasing. Accessibility of mouse mutants is one of the major rules of EMMA. By having a constant renewal of mouse lines, the most important strains are available when needed, with exportation times reduced to the minimum. The work carried out by EMMA represents a crucial element in achieving the potential benefits for human health genetic research. It has already become clear that EMMA is having an influence on parts of biomedical research in Europe, strengthening the competitiveness of the European research area.

Acknowledgments

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Reference

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A model for genetic standardization: The Jackson Laboratory's Standard for genetic stability

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We will discuss two sub-strains of the same parent mouse and define the differences between them: C57BL/6J and C57BL/10J.

The definition of a substrain is

- Colonies separated by more than 20 generations from the progenitor colony. (if you get a strain from a progenitor colony, while your colony is increasing 10 generations, so too is the progenitor colony so after 10 generations or 2.5 years, they are really 20 generations apart and need to be designated a sub-strain).
- Colonies are genetically distinct
    - Residual heterozygosity at time of separation
    - Spontaneous mutations that are fixed
    - Genetic contamination
    - Deliberate outcrossing for experimental purposes
- Colonies are maintained completely independent of the progenitor strain

The nomenclature for a sub-strain is

- A sub-strain is identified by appending a "/" followed by a line number (optional) and the Lab Code of the holder to the root strain name.
- Sub-strains are designated by the addition of the Lab Codes of subsequent holders of the strain, without another forward slash.
- Lab Codes should be accumulated because genetic changes continue to occur over time (this is a recent nomenclature rule change).

In the example of C57BL/6JOlaHsdEi, C57BL = parent strain designation, 6JOlaHsdEi = sub-strain designation, 6 = line number, Ola = lab code, Hsd = lab code, Ei = lab code

Genetic contamination.

Causes of genetic contamination are:

1. the accidental introduction of undefined genetic material (no longer truly inbred – loss of homozygosity) leading to genetic variability or undefined "novel" stock
2. not accidental – but records are lost in time or forgotten
3. the direct mix up of two distinct but genetically different strains, e.g. same coat color.

The effect of genetic contamination is:

1. genotype is not the equal to phenotype
2. not reproducible by others
3. incompatible with previously obtained data
4. irrelevant or misleading
5. confusion.

Genetic contamination can be detected by tail skin grafting, isoenzyme markers, observation by trained technicians to identify phenotypic deviants, and SNP markers.

SNP markers to detect contamination.

The majority of strains can be distinguished by 3 or more SNP's. SNP markers can distinguish more than 120 different mouse strains (see Petkov, *et al.* (2004). A SNP assay sees only 1-3 bases so therefore they monitor only about 300 bases. Similarly, MIT markers monitor only a small region of DNA. 28 SNPs or 100 MIT markers are only capable of detecting gross genetic contamination, not genetic drift.

Genetic drift.

Genetic drift is the mechanism of evolution that acts in concert with natural selection to change species over time. It is the constant tendency of genes to evolve even in the absence of selective forces. It is the statistical phenomenon that results from the effect of chance on the birth, survival and reproduction of individuals, and it is most prone to be seen in small populations.

What is it? It is random change. The mutation rate is 10^{-5} to 10^{-8} and the allele fixation rate is not known.

Does it matter? It probably does. Which of the 2.55 x 10^{9} nucleotides of the mouse genome do you depend upon for continuity of your work?

Why is the idea of genetic drift becoming important?

1. the human and mouse genomes have been sequenced
2. the pace of scientific inquiry is increasing and the very nature of biological science is changing
3. projects are becoming considerably larger and more costly
4. the depth and complexity and cost of the knowledge obtained is many orders of magnitude greater than envisioned only a few years ago. Data has to last and it has to be relevant over time.

Genetic stability

With genetic stability we cannot stop change from happening but we can slow it down.

One method is the use of a foundation colony and subsequent expansion and production colonies from the founders. The founder colony is slowly increased in generation number over time. The slower the better.

You can avoid creating sub-strains by frequently replacing the colony with littermate breeders from the progenitor colony. If it is intended to maintain a colony for more than 2 or 3 generations, do not establish the colony from non-littermate mice from production level stocks. Colony maintenance to be done correctly, is very difficult and requires much attention to detail. Technicians responsible for the colonies must be well trained to give special oversight to the breeding colonies.

Genetic stability can be controlled with a good quality control program. Such a program consists of:

1. the identification of any phenotypic deviants and their removal from the colony
2. frequent and regular genetic testing of breeding stocks using biochemical, SNP's and SSLP's
3. maintenance of strain characteristic databases.

Colony pedigreeing, avoidance of sub-strains, and genetic quality control are all highly effective in limiting genetic drift and have worked well for 75 years. Now we are able to use a genetic stability program. Such a program replaces the foundation breeders using cryo-preserved embryos at frequent intervals.

Minor technological improvements have now made it cheaper and easier to freeze embryos. Before it was so costly that embryos were only frozen and recovered as needed, usually in the case of disasters. Now, because of the decreased costs, it can be used for colony maintenance.

Why reinitiate from frozen embryos now? With projects like the Phenome Project and sequencing C57BL/6J, it is important that the genome be stabilized with the genetic stability program.

What defines the differences between C57BL/6J and C57BL.10J? First of all, the nomenclature. Next the origin, the history, the strain development path, and finally, the genetics. In the panel of Jackson's 2000 SNZP markers, there are 28 identified differences (2.4%). In the Jackson panel of 25 isoenzymes there are no differences. There is a known difference in erythrocyte antigen, Ea9. Note: these are identified differences. The unknown differences can be significant!

A pragmatic approach to genetic background problems in the analysis of genetically modified mice

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Increasingly sophisticated and precise molecular genetic tools are applied to mice in order to study the cellular mechanisms underlying higher brain functions, including learning and memory. However, despite such advanced technology several studies have produced unclear or conflicting results. One reason for this is that genetic background and environment alone produce sufficient variation to span the range of behavioral variables in many tests and can easily mask or fake mutation effects if genetic studies are not designed properly. Thus, mutation effects can only be contrasted statistically against the influences of genetic background and environment. In most situations, this is most efficiently and reproducibly achieved if

(i) mutations are backcrossed to and maintained in one or (preferably) two well-characterized, commonly available inbred strains and

(ii) if mutant and wild-type littermates are analyzed on a well defined genetic background that can be reproduced at any time from the inbred stocks.

This may be inbred mice, F1 hybrids or a F2 generation, depending on the genetic model and the hypothesis being tested. However, these recommendations do not eliminate the so called 'flanking allele problem' , genetic bias resulting from genetic linkage between the targeted locus and neighboring genes. If desired, such bias can be removed using simple modifications of the standard breeding schemes.

Playing with the genetic background to modulate the phenotype of a mutation: the example of a mouse model of erythropoietic protoporphyria

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The ferrochelatase deficiency mutation of the mouse, which arose in a genetic background very close to the BALB/cByJ inbred strain, after mutagenic treatment with ethylnitrosourea, is a good model for human erythropoietic protoporphyria. Mutant mice exhibit photo-sensitivity, jaundice, enlarged abdomen (due to hepatomegaly and splenomegaly) and anaemia. While producing congenic strains by repeatedly backcrossing the mutation with the BALB/c, C57BL/6J and SJL/Orl inbred strains, it became rapidly obvious that the severity of the phenotype was strongly dependent on the genetic background. Mice of the three congenic strains were submitted to phenotypic analysis at various ages, to characterize the haematological, biochemical and histological features associated with each strain. From the original model of erythropoietic protoporphyria, we have developed three models of protoporphyria which better reflect the variety of phenotypes observed in man. These models will help to undertake a genetic study with the aim of identifying, by a QTL approach, genomic regions controlling these differences.

Is there a need for a genetically standardized background in animal models? Implications on biomedical research

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The genome projects and the possibility to genetically modify rodents have not only broadened our knowledge about the number of structural genes it has made more scientists aware of the fact that phenotypes often depend on the genetic background of a strain under study. This finding along with the knowledge that the number of genes is much less than originally envisaged (~ 30.000) implies that the variant phenotypes detected in congenic strains carrying the same mutation are mostly the result of modifier genes.

Interestingly, it is not a new insight gained from gene targeting that there are background effects on the phenotype of mutants. There are quite some good examples dating back some decades such as acholuric jaundice (Ugt1) which acts as a lethal on ACI but not on RHA background, or fatty-corpulent ($Lepr^{fa-c}$) which displays different metabolic disorders on SHR and LA background. This information as well as that gained from genetically modified genes undoubtedly calls for the introgression of spontaneous, engineered and induced mutations into different genetic backgrounds rather than its maintenance on a undefined segregating background or even on a single inbred background. Analysing such differences in phenotypic expression will provide insights into developmental pathways critical to fundamental biological processes and into the pathophyiology of mono- and polygenetically controlled disease processes.

Recent data from studies analysing $Il10^{tm1Cgn}$ on different genetic backgrounds will be presented. These facts do actually call for introgressing any interesting new (engineered or induced) mutation into more than one suitable inbred background. Phenotyping a broad range of inbred strains will thus provide the basis for dissecting the effects of modifier genes once transferred onto these backgrounds and to understand the extreme variation sometimes seen in so-called monogenetically controlled diseases. We have, however, to keep in mind that many of the common inbred strains obtained from different sources may carry unknown mutations that will affect response patterns in the animals studied. A few examples for this are, e.g. 129P and 129X derived strains that are visually impaired, or C57BL/6JOlaHsd mice with Î±-synuclein ablation ($Snca^{m1}$), F344/DuCrj and some Crl or Hsd colonies of F344 being defective in Dpp4 (CD26). Either of those afore mentioned defects will more or less lead to different functional/behavioural phenotypes. Unfortunately, quite a many scientists who have accepted that it is important to study mutations in the context of a defined background do not provide details on the origin of their congenic strain, nor on the number of (effective) backcross generations. It is therefore inevitable that results obtained from such strains may bear (major) consequences for the particular phenotype under investigation. Although consequent genetic monitoring programs will minimise genetic drift the fixation of mutations that escape detection in phenotyping programs cannot be prevented. It is thus important that we all adhere to the nomenclature rules set forth by the International Committee on Standardized Genetic Nomenclature for Mice and the Rat Genome and Nomenclature Committee and that mutations that have been detected be reported immediately. Furthermore measures should be taken to eliminate such variants rather than to maintain these colonies segregating. This applies especially for commercial breeders.

In this context it should be mentioned that the environment as well as RNA genes in the non-coding sequences, the degree of DNA-methylation and imprinting may also modify the phenotype.

Strain differences in response to anaesthetics and analgesics in the rat

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Previous studies have demonstrated significant strain differences in the response to anaesthetics in the rabbit. In order to determine whether strain differences in response to anaesthetics and analgesics also occur in the rat, eight frequently used inbred strains (ACI, BN, COP, F344, LEW, SHR, WAG and WKY) were selected. These strains (n=6 males/strain) were each injected intravenously with two different analgesics (buprenorphine, 0,05 mg/kg and nalbuphine, 1 mg/kg) and three different anaesthetics (propofol, 10 mg/kg; ketamine, 25 mg/kg and medetomidine, 50 Âµg/kg). The dosages used were based on literature reviews. The response to the analgesic was measured by using the tail-flick test. The response to the anaesthetic was defined as the interval between loss and regain of righting reflex. Buprenorphine exhibited large interstrain variation with the ACI (high analgesia) and the WKY (low analgesia) being the most divergent strains. With respect to nalbuphine strain differences were not detected. The COP had the lowest response to propofol and the F344 showed the highest response. Ketamine induced a severe respiratory depression in the ACI and BN strain. Medetomidine did not induce a loss of righting reflex in the BN rat. Future studies will focus on the genetic background of the differences in response to anaesthetics and

Pain and Distress Management in Chronic Disease Models

The knowledge of nociception mechanisms and of the interactions between stress and pain can help the development of animal models for chronic pain study

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Pain is necessary for survival, but persitent pain can result in anxiety, depression and severe reduction in welfare and quality of. The discriminative and affective dimensions of pain are both thought to be regulated in an activity-dependent fashion. Recent studies have identified neurons and molecules that regulate sensitivity and the parallel pathways that distribute nociceptive information to limbic or sensory areas of the forebrain.

The presentation will focus on i) salient cellular and neurobiological consequences of pain, especially those involved in the generation and maintenance of chronic pain, ii) the relationship between stress and sensitivity to nociceptive stimuli, and iii) the interactions between inflammatory events (i.e induced by nerve injury) and the modulation of nociceptive informations. Pain can be considered as having a sensory (discriminative) and an effective (unpleasantness) dimension. Chronic pain states can lead to secondary negative effects such as anxiety and depression. Neurophysiological evidences indicate that parallel spinal pathways simultaneously distribute information to brain circuits involved in either sensory or affective dimension of pain. The spinothalamic tract is considered to be mainly involved in sensory discrimination qualities of the stimulus ; it originates primarily from neurons located in the neck of the dorsal horn of the spinal cord and terminates within the ventroposterior and ventrobasal thalamus before projecting to the cortex. The second pathway is more extensive, it derives from lamina I neurons in the dorsal horn where NK1 receptors are expressed ; it is more involved with signalling the emotional intensity of pain than with discriminative nature of the stimulus. This second pathway terminates within the parabrachial nucleus and the periaqueducal grey, two nuclei respectively involved with emotional and 'information-gating oriented' responses. These nuclei in turn project onto structures such as hypothalamus and amygdalia that modulate the affective dimension of pain and control autonomic activity involved for instance in inflammatory responses. In case of selective destruction of these neuronal populations, the increased sensitivity to stimulation that follows inflammation or mechanical manipulation of peripheral nerves is lost. The connections of lamina I neurons towards amygdalia via the parabrachial nucleus is likely to provide a substrate for the development of secondary effects of lasting pain such as anxiety or depression. Some specific examples of pain modulation by stress or inflammation will be presented under the scope of studying animal models used to alleviate chronic pain.

Continuous monitoring of corticosterone in freely moving rats in combination with automated blood sampling for anxiolytic drug screening

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The HPA axis describes the relation between the hypothalamus, pituitary gland and adrenal gland in response to stress. One of the hormones produced by the rat adrenal cortex in response to stress is corticosterone. The accepted means of monitoring corticosterone in a rat requires the collection of whole blood samples of at least 0.2 mL in order to produce 0.1 mL of the blood serum required for a radioimmunoassay. Repeated blood sampling for the purpose of monitoring serum corticosterone will limit opportunities for analysis of other chemicals in the blood, since there are restrictions to the total blood volume that may be removed without comprising the animal. For that reason, several animals are usually required when screening new drug candidates for possible anxiolytic properties. In this presentation, we describe a new method for monitoring both corticosterone and the disposition of a drug in the same awake and freely-moving rat. This approach reduces the number of animals needed, and refines the study by providing more effective correlations. In addition, we describe a new analytical technique for corticosterone based on LC/MS/MS as an alternative to RIA or ELISA methods of analysis. In this approach, an in vivo ultrafiltration probe is implanted subcutaneously to provide filtered extracellular fluid collected continuously from the subcutaneous tissue. At the same time, blood is removed at programmed intervals by an automated blood sampling device for pharmacokinetic analysis. Both sampling methods permit the animal to move without restraint and associated stress. This approach is used to develop an animal model for anxiolytic drug screening by correlating the disposition of the drug (in the blood) with changes in corticosterone (in the ultrafiltrate) in the same animal at the same time, in response to an external stressor (noise). Comparisons between serum corticosterone and ultrafiltrate corticosterone establish the validity of this sampling method. Diazepam is used as one example of an anxiolytic drug utilized in this screening model, and saline is a non-anxiolytic control. The automated blood sampling device also permits automated intravenous drug dosing so that the administration of the control or drug can be accomplished without handling and associated dosing stress. Finally, correlations are provided between the animal's activity (clockwise vs. counterclockwise rotations and rearing) which is also recorded by the blood sampling device.

Clinical management of the systemic 1-methyl-4phenyl-1,2,3,6 tetrahydropyridine (mptp) animal model of Parkinson's Disease in nonhuman primates

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Parkinson's disease (PD), first described by James Parkinson in 1817, is a neurologic disorder characterized by resting tremor, rigidity, bradykinesia , and postural instability. The hallmark pathological finding believed to underlie these symptoms, is the degeneration of dopaminergic neurons of the substantia nigra pars compacta. Agents that selectively disrupt or destroy catecholaminergic systems, such as reserpine, methamphetamine, 6-hydroxydopamine and 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine, commonly known as MPTP, have been used to develop PD animal models. Since its introduction in the early 1980s, the MPTP-treated nonhuman primate has become an extremely valuable model for this disorder. Unilateral intracarotid injection of the MPTP toxin produces focal lesions of the dopaminergic system. This treatment protocol generally results in clinically less invasive parkinsonism. There are three main disadvantages of this treatment modality: 1) it may result in some non-parkinsonian features, 2) it is not particularly valuable in the study of dyskinesia, one of the most debilitating side-effect associated with conventional dopamine therapy for PD, and 3) near-complete recovery of motor functions is common in these animals. Systemic injections of MPTP can result in a more faithful reproduction of the biochemical and behavioral phenotype of parkinsonism in humans. However, maintaining bilaterally lesioned nonhuman primates can be challenging since it has proven extremely difficult to induce a stable moderately parkinsonian state with this method of treatment. In this presentation we will detail the daily clinical management of acute and chronic systemic MPTP monkeys, including supportive care and drug therapy, using a collaborative approach between the research laboratories and the veterinary staff. We will also describe the Emory University Institutional Animal Use and Care Committee guidelines for the use of MPTP including well defined endpoints. Our ultimate aim is to optimize animal care and decrease animal distress inherent to this MPTP animal model, a 'gold standard' model to study Parkinson's Disease. {Supported by the NIH base grant of Yerkes Primate Center}.

Pain and distress management of swine with surgically produced diseases

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When using swine as animal models it is frequently necessary to produce a disease condition surgically, implant biomechanical devices that may cause anatomic and physiologic defects or perform major surgical procedures that are prone to complications. Laboratory animal veterinarians have the responsibility of ensuring that the long-term aftercare of these animals is appropriate and minimizes animal pain and distress without compromising the goals of the research. Minimization of pain and distress requires appropriate monitoring and husbandry procedures as well as the use of pharmaceutical agents.

The workshop will present information on appropriate husbandry, nutrition, behavioral observations and clinical care of swine before and after the animals are compromised surgically. Specific disease conditions will be covered including: heart failure models (pressure overload, volume overload, dilated cardiomyopathy), myocardial infarction, organ transplantation, fetal cardiac and urologic surgery, arteriovenous fistulas and shunts, cardiopulmonary bypass procedures. The workshop will also cover implantation of devices such as stents in the cardiovascular and biliary systems, pacemakers and chronic intravascular catheterization procedures.

The disease conditions will be discussed in terms of proper protocol design. Included in this discussion is the necessity of customizing anesthetic, analgesic and perioperative care procedures. Intraoperative monitoring, long-term care and clinical evaluation of the animals will be discussed. The importance of cooperative interactions between investigators and the veterinary staff, as well as flexibility in design of the research protocol will be emphasized. Following the formal presentations the workshop presenters will have a practical problem solving session for protocols submitted by the audience.

Evaluation of bioethical aspects and animal welfare in colorectal metastatic models

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The welfare term should be important and well-established as a part of any modern laboratoryanimal science, and relevant humane endpoints should be identified, described, and incorporatedinto the experimental protocol. Humane endpoints refer to the decision points at which animalsmust be euthanised, in order to avoid unnecessary suffering.From a bioethical perspective it is also very important to find a scientific basis for humaneendpoints; killing the animals at a point of time where they are not really suffering, may lead tostopping experiments at a time point which is too early, leading to the loss of generation ofvaluable data and will increase the number of animals used unnecessarily.In a literature study of colorectal liver metastatic models, we found the body weight as the onlyparameter registered to evaluate the animal welfare. However, this has proven to be a veryunreliable indicator of the animal conditions and welfare. Therefore, the goal of the present studyhas been to find other biomedical parameters which could be used to define humane endpoints.Furthermore, this could provide useful guidance and criteria for determining when animals havedeveloped liver metastases.In the present study, a rat model of liver metastases was induced by intraportal injection of colonadrenocarcinoma cell line cells (CC531) in syngenetic WAG rats. After the injection of tumourcells, the rats were observed intensively and frequently in order to find possible predictive clinicaland/or other parameters that could be used when defining new humane endpoints.The parameters evaluated in the present study were:

-the overall clinical condition; including appearance, posture, behaviour and physiological responses.
-the body weight. -the liver enzymes (e.g Alanine aminotransferase, Alkaline phosphatase, and Aspartate aminotransferase) in the blood.

To be able to define humane endpoints of this model, it is necessary to have an accurate way to correlate tumour development and parameters used for defining new humane endpoints. We found that the laparoscopic examinations of the peritoneum is a very simple as well as accurate method for measurement of parameters used for defining the degree and size of liver metastases, and thereby a good method for defining humane endpoints. In future studies we wish to investigate the influence of the liver metastases on the overall physiology of the animal. By using a telemetry-based monitoring system and video recordings, we intent to investigate following parameters: behavioural activity, body temperature, heat rate, and blood pressure. Hopefully these studies will reveal new precise parameters indicating the presence and degree of metastases and can be implemented as new humane endpoints.

Classification of animal suffering – how useful are current grading schemes?

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Summary

A number of countries including the UK have schemes for classifying of the level of suffering experienced by animals undergoing scientific procedures. Such schemes allocate a 'label' – a number, letter, or word (e.g. mild, moderate, substantial) – to different degrees of suffering, usually at the level of the study protocol/procedure and/or the overall project. These schemes can have several purposes. They can help in assessing levels of suffering and in encouraging refinement, assist in carrying out a cost/benefit assessment, provide a project management tool, and act as a means of providing information to the public. This paper summarises a project carried out by the RSPCA and the Boyd Group, which brought together a diverse group of stakeholders to consider whether severity classification schemes are useful and appropriate for each purpose, and to propose some principles for improving on existing schemes.

Key words: severity classification, animal suffering, harm-benefit assessment

Introduction

In the UK, the way in which the severity of scientific procedures on animals is classified under the Animals (Scientific Procedures) Act 1986 has been a subject of discussion and debate for several years (see Home Office 2000 for a brief description of the scheme). The classification was identified as an issue of concern – and considerable confusion – when the Government's independent advisory committee on animal procedures, the Animal Procedures Committee (APC), carried out a public consultation on the cost-benefit assessment that underpins the legislation (APC, 2003). The classification of severity was also one of the key issues for the Technical Expert Working Groups (TEWGs) set up to provide advice to the European Commission on the review of Directive 86/609 (TEWG Cost-Benefit 2004) and was further considered in a recent FELASA survey of ethical review processes within FELASA member countries (FELASA, in press – *see Abstract in this volume p.*).

The classification of severity is a subject that interests and affects many different stakeholder groups, including the RSPCA, which has longstanding concerns regarding this issue, and the UK Boyd Group. The latter is a discussion forum which brings together people with a wide range of perspectives and expertise relating to the use of animals in research and testing. The authors therefore thought it would provide an ideal forum in which to progress discussion of the nature and purpose of severity classification. This paper summarises these discussions. The full report (Boyd Group/RSPCA 2004) is available from the address above and via the Boyd Group's web-site *(www.boyd-group.demon.co.uk)*.

Existing grading schemes

Many countries have some form of scheme within their regulatory system for classifying and recording levels of animal suffering, and these adopt a variety of approaches (see FELASA in press for examples). In general, between three and five 'levels' of suffering are identified, and categorised by descriptive terms (e.g. mild, moderate, substantial), numbers or letters. Some schemes categorise the severity of individual protocols or procedures; others categorise the overall severity of a project. The classification may refer solely to the level of suffering caused directly by the experiments or other procedures, or may cover the lifetime experience of the animal, taking into account factors such as the source, transport, husbandry and care of the animals, and restraint and identification procedures. Such schemes may be used prospectively, to describe the level of suffering expected to be caused to the animals in the procedure or project, and/or retrospectively, to describe the level of suffering actually experienced by the animals used (or a proportion of them). The categories may refer to the maximum severity expected or experienced by individual animals, and/or to that caused to 'the average animal'. Rather little guidance is available on how to assess and appropriately categorise levels of severity.

Is there potential for harmonisation of existing schemes?

The topic of this FELASA Symposium is Internationalisation and Harmonisation, so it is highly pertinent to consider whether, given the diversity of approaches to severity classification, any one system is 'best', and, if so, whether existing systems would benefit from being harmonised. This will require consideration of all of the factors and variations listed in the preceding paragraph and, importantly, should include not only how the severity classification system is applied, but also how the resulting severity labels are *used in practice,* particularly when 'weighing' harms and benefits as part of consideration of the ethical acceptability (or otherwise) of using animals.

Aim of the Boyd Group/RSPCA project

The Boyd Group/RSPCA project set out to consider how the UK severity classification scheme operates in practice, but it was quickly realised that it was necessary to take a step back and ask the more fundamental question noted above, that is: are classification schemes such as this useful and appropriate for their intended purpose or purposes, whatever these may be?

Three groups of stakeholders were consulted on these points in separate working groups, comprising: (i) senior animal technologists and veterinarians; (ii) scientists using

animals; and (iii) representatives of animal welfare and antivivisection groups. Each group was asked to define the *purpose* of severity classification, consider *who* uses it, *how* they use it, and whether it is a *valuable tool* in practice. They were also asked for their views on the nature of the classification process, for example, whether terminology is important, where the difficulties lie, and how the process could be improved in practice.

In all three groups, the discussions were extremely constructive, allowing a range of perspectives to be brought to bear on the key questions. There was a great deal of concordance between the groups despite differences of opinion on the ethical acceptability of the use of animals in general. A summary of some of the main points is provided below.

Purposes of severity classification

It was agreed that the severity classification has two main purposes, in that: (i) it can be used to provide public information on the harms to animals in research and (ii) it has value as a practical tool to assist in managing animal suffering and in carrying out harm-benefit assessment.

Severity classification as a public information tool

Most participants in the discussion groups agreed that, for openness and transparency, it is important that information on the harms and the benefits of animal experiments are reported in the public domain. However, a number of difficulties were identified in the use of severity classification as a public information tool.

A particular concern arises where severity categories are labelled with 'value-laden' terms such 'mild', 'moderate' and 'substantial'. There are very different perceptions of what such terms mean. For example, it was suggested that the 'average member of the public' would be surprised that abdominal surgery, albeit with appropriate pain management, is categorised as anything but a substantial procedure, yet some surgery would be classed as moderate in the UK. The problem is compounded when *categorisation* of severity is confused with *recognition and assessment* of suffering, and therefore differences of opinion such as that described above are taken to imply that suffering has been underestimated and therefore has not been relieved in practice; and furthermore that any harm-benefit assessment based on the categorisation is 'wrong'.

All three discussion groups agreed that data for public information that are based on a prediction of what animals *may* experience and/or which 'average out' suffering between several animals (rather than providing a measure of the impact on individual animals) are questionable as a true guide to levels of suffering caused in practice and therefore are misleading.

Practical uses of severity classification

It was agreed that the *process* of classifying severity helps people think more carefully about levels of suffering and encourages refinement generally; and that the resulting *categories* can:

(i) help to define clear upper limits on animal suffering, and therefore assist in implementing humane end-points; and

(ii) identify techniques, procedures and protocols that cause the most animal suffering, so that these can be prioritised for application of the Three Rs, and highlighted for additional review.

Such classification therefore provides a tool to assist in managing severity within scientific projects and also contributes to the harm/benefit evaluation.

There was a great deal of consensus amongst all three groups that these practical outcomes are very beneficial – but that the actual classification is hard to do. It can feel arbitrary, particularly when there is little guidance on what factors to include (e.g. just the effects of procedures, or of procedures and aspects of husbandry and care) and how to weight each of these factors in the overall assessment, as well as little information to assist in identifying the nature and level of suffering experienced by the various different species of laboratory animal. It was also noted that it can be particularly hard to assign severity categories when the adverse effects of a particular procedure are uncertain or unpredictable. One group suggested that difficulties can also arise where severity classification is an integral part of the regulatory system and there is variation in how it is applied by regulators.

It was emphasised that the categories should encompass *all* potential adverse effects – psychological as well as physical. Moreover, with particular reference to the terminology, it was widely agreed that descriptive word labels are more effective in focussing attention on the need for refinement than number labels. However, one group argued that severity labels 'mild', 'moderate' and 'substantial' are too pain-related and do not adequately reflect other kinds of adverse effect, such as stress, anxiety, and other more specific effects, such as nausea; and many participants considered the term 'moderate' to be too comfortable and broad a category, which became the default position.

Some principles

Each of the three working groups defined some principles for an improved severity classification system, resulting in strong consensus between all three groups. It was agreed that severity classification should:

- focus on the individual, not an 'average', animal
- be assessed from the animal's point of view
- be based on the total impact of the scientific work on the normal wellbeing of the animal
- adopt a 'holistic' approach, in which there is an attempt to consider all factors that can potentially influence well-being, including psychological/ emotional effects (e.g. anxiety, fear, boredom) as well as physical effects, and their *duration*; and wider factors, such as transport and husbandry (either routinely, or when these differ from the norm) as well as the procedures themselves
- use descriptive terms, which should encompass stress, anxiety and nausea as well as pain, rather than numbers
- when used prospectively, reflect the probability that the effect will occur in practice.

For the UK system specifically, it was generally felt that the category 'moderate' (or its equivalent) should be subdivided.

In addition, from the point of view of providing public information, there was a general consensus that a system of *retrospective* reporting of *actual* suffering is needed. Information on the nature, degree and duration of suffering,

together with an estimate of the number of animals in each category is required. Furthermore, it was strongly argued that the reasons for the study should also be explained, in order to put the harms to animals in context. It was agreed that it is difficult to satisfy these requirements by publishing statistics of animal use alone, and that publication of appropriate lay summaries along side the statistics could help in this.

Future work

In order to achieve any of the above goals there is a need for more information on the recognition and assessment of animal suffering, both physical and psychological. FELASA published guidance on the recognition of pain and distress in rodents and rabbits over ten years ago (Baumans *et al.* 1994). It was agreed that this needs updating and similar guidance developed for other species, drawing on other existing guidance, such as that used in Switzerland (Swiss Federal Veterinary Office (undated)).

It was further agreed that there is an urgent need for more guidance on how to assign the different severity categories, preferably with worked examples. Retrospective review of actual versus predicted suffering would also help refine the categorisation process. Lastly, there needs to be a more descriptive way of presenting retrospective data on suffering for purposes of public information. This is an issue that the UK APC is currently working on.

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Training and International Recognition

FELASA Accreditation of education and training programmes

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FELASA has established an accreditation system for teaching programmes according to the 4categories for which FELASA has published guidelines previously. The new system wasintroduced 1 January 2003.This quality assurance system is intended to assist in the development of uniform high qualityeducational programmes for laboratory animal technicians (category A1-A4), research technicians(category B), scientists (category C) and specialists (category D) throughout Europe.One of FELASA's main activities is to drive the process of continuous implementation ofrefinement in the husbandry, use of animals in research as well as design of animal experiments,which goes hand in hand with good science. FELASA trusts that professional competence of allstaff working with animals is a prerequisite for implementation of the Three Rs and for highquality science. Consequently, the establishment of an accreditation system ensuring high qualityeducation in laboratory animal science is seen as an important milestone for FELASA.The review process is carried via email communication. No paper documents are circulated. Theprocess is conducted and maintained in strict confidence. Further details on the process can befound in the Recommendations published in Laboratory Animals' October 2003 issue.National liaison experts may be consulted and take part in the review process. The FELASA Board has appointed national liaison experts ensuring that all geographical areas are covered.

Application Procedure: The application form can be downloaded from the FELASA web sitewww.felasa.org. All applications including all documents to be assessed by the Board must besubmitted electronically to the chairman.

Update on the European College of Laboratory Animal Medicine

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The European College of Laboratory Animal Medicine (ECLAM) has finalised its review for applications for de-facto ('grandfather') status. After detailed review 66 de-facto Diplomats met the criteria set down by our Constitution and the European Board for Veterinary Specialisation (EBVS):

- -have at least seven years of experience in the speciality;
- -spend at least 60 per cent of his or her time in the speciality;
- -have published at least three original articles in refereed journals as first author and at least three additional articles as co-author excluding non-peer reviewed review articles and proceedings abstracts;
- -be significantly active in Europe in the speculate of Laboratory Animal Medicine. These 66 de-facto Diplomates join the 7 Founding Diplomates to give a total of 73 Diplomates for the new College.

ECLAM has also taken active steps to complete formalising the structure of its College, allowing to function under its Constitution and Bylaws, as requested by EBVS. Actions include establishing a Training Committee charged with the evaluation of training program applications, recruitment of a Credentials Committee and an Examination Committee.

There are 7 Training programs, based around Europe, according to EBVS rules, these approved training programs and their supervisors will have to be re-evaluated every five years.

An Examination Committee will describe the nature and scope of the examination, evaluate the material used for examination, grade the examination and determining passing points, and to physically supervise the examinations.

The Credentials Committee will define the most time-efficient pathway to qualify for examination, establish a standard procedure by which the prerequisites for submission to examination are made, and evaluate whether candidates have met these criteria - by formal training or the alternative route by experience - to be given permission to take the examination. It will review continuing education requirements.

Harmonisation of the European Academic Socrates programs concerning the practice in experiments using animals academic harmonisation on animal experiments

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Summary

The SOCRATES programme supports European cooperation from school to higher Education. The higher education sector established in 1987 is "the ERASMUS programme" which promotes the knowledge for new competencies and qualifications through teaching in an European context.

The programme is open to all levels of higher Education study including the doctorate, and promotes mainly the physical mobility of students and their teaching staff. We propose a specialty to accompany the body course of scientists, chemists and veterinarian which allow to adapt in an ethical context, the practical teaching using animals for new competencies and qualifications of European students.

The Erasmus Programme

The SOCRATES programme supports European cooperation in education. It is subdivided in smaller programs. One of them, "the ERASMUS program" concerns the higher education and begun in 1987. It is open to Universities and other higher Education public institutions and is devoted to students from the entry at University up to end of the doctorate. It is based on students and teachers exchange in Europe. The duration of the exchange are from 3 months to one year for a student and of 7 days for a teacher's courses. To make easier the exchanges, it was decided to harmonize the courses in the different European countries on the basis of a new degree system, which is progressively organized in all the universities of Europe. It refers to the three following levels: Licence (3 years from the entry at the university), master (2 years after the licence) and Doctorate (3 years after the master). So, it is named by its initials: The LMD system.

The Erasmus program develops progressively with time as indicated by the statistics. In 2002/2003, the program involves 30 European countries, 1 800 universities or higher education institutions, 12 000 teachers , and 124 000 Erasmus students. Among them,12 000 are studying in biomedical and natural sciences. The mean duration of the mobility for a student is of 7 months, and for a professor of a few days to organize the student mobility and to teach or give lesson.

To plane an exchange, the procedure consists in a statement of a bilateral agreement for 3 years between 2 universities or institutions of two different countries. Most often, the cooperation arises by contact in search between two professors working on a same subject area. Then, the bilateral exchange between students becomes possible. In each university, the course is divided in units or modules. Some of them are obligatory (or imperative), the other are optional (adding units). Each unit has a credit value corresponding to the quantity of work to furnish by a student. A unit with its corresponding credit is named an ECT unit. On the basis of the European Credit Transfer System (ECTS), it is possible for a student to move to an host country for an academic semester and to study a part of his course composed of units which have to be chosen by comparing the programme and the credit in the home university and in the host universities. The credits of the units obtained in the host country are added to those obtained in the home country. Their validation occurs in the home university. The examination is successful whether the whole program has been studied and whether the student has obtain enough credits. A sum of 30 credits is needed for each semester.

The objectives of the Erasmus program are ambitious as shown by few statements:1) European harmonization was stated by France, Italia, Great Britain, in Paris in 1998; 2) Europe is a space for high education level, as proposed in Bologna in 1999; 3) Europe must reach the first place for knowledge in the world, as announced in Lisbon in 2000; 4) A high quality for the educational system was commanded in Stockholm 2001. As a result, a final objective is to develop trans-national curriculum for new competencies and qualifications. It is also to stimulate public and private trans-national cooperation activities.

The harmonization in experimental sciences using animals.

The ambitious objectives interest also education in experimental sciences using animals. Three convergent conditions meet now to promote the harmonization and adapt the education to offer the best formation to our students enrolled in courses of experimental sciences using animals, for physiology course, veterinarian course or physician course. The conditions are 1) the new degree system in Europe, 2) the knowledge for new competencies and qualifications, 3) the revision of the European Directive on experiments using animals. The actual realty is that the courses in physiology, pharmacology and surgery use different animal species, mainly rodents, rabbits and amphibians and also pigs, sheep and fishes. The experiments may be non invasive or when they are invasive, the animals are anaesthetized and euthanazed without weakness. The protocols are generally submitted to an ethical committee.

In the new system with the ambitious objectives, we propose to reinforce the body of courses offered in biology and physiology by an optional specialty (table I) entitled "knowledge and ability for using animals in scientific experiments " (connaissances et savoir faire pour utiliser l'animal dans l'experimentation scientifique) "based on few principles:

- to be gradual on 3-5 years (benefit of the interaction with normal course)
- to use animals late (at the last step of the specialty) to save animal life and for efficacy
- to be an accompaniment to the "Animal biology and physiology course"
- to be adapted to accompany other courses

The specialty content could be divided in 4 ECTS units or modules, the duration of each being of 24 hours: 1) Mastery with understanding and control of the experimental science including ethology of the laboratory animal, the principles of the experimental method ("la méthode expérimentale ") and the mastering of the technology and the results validity ; 2) legislation (experiments, animals, housing, risks….); 3) ethics (the experimenter and his practice and manner) and animal well being; 4) mastery and management of the practical experiments (protocols, procedures). An example of animal biology and physiology course with specialty for biologists is shown in table II. The benefit could be maximal by a progressive education from the second year of licence up to the end of the master. An advantage is that the students have already the knowledge at the entry in doctorate (or in another professional activity) to manage the experimental protocol and its context (ethic, legislative and scientific).This is essential for the ethical principles, and for the scientific stake. The practice in the specialized field during the doctorate offers the possibility to become a specialist.

Pedagogic aim in the European context

Our proposal takes into account all the FELASA recommendations which are the best basis.

It also takes in account the pedagogic aim of high competences for students reaching the level of master or of doctors. At this high level, it is very powerful to dissociate mental reasoning and practical training to prepare correctly the students to be responsible and aware of the conceptualisation of an experimental protocol and of a practical work.

As a conclusion, to answer to the scientific stake, the best formation requires two components: one concerning the thinking with criticism and questions to develop the ethical principles, and the second corresponding to the movements and motor control to conduct a protocol in the legislative context and good practice. Such a formation is necessary to offer to the students the ability to do good science in the respect of the well being of the animals.

Degree	LMD course	Specialty (4 ECT optional units)
D3 (year 8)	Doctorate	Specialisation
D2 (year 7)		
D1(year 6)		Training by specialized search
M2 (year 5)	Master	Management of the practical experiments
M1 (year 4)		Ethics and animal well-being
L3 (year 3)	Licence	Legislation
L2 (year 2)		Experimental science
L1 (year 1)		

Table I: Proposition of the organisation of the specialty in accompaniment of the animal biology and physiology course in universities. The three column show from left to right , the degrees of qualification in preparation, the level of the course, the program of the units in the specialty. L,M,D indicate the level of qualification to obtain (Licence, master and doctorate), 1,2,3 indicate the year in the level, the corresponding year from the entry in the university is indicated in brackets .

Level	General Content
Unit 1 (L2)	Experimental science (understanding and control): - Ethology of the laboratory animals, - Principles of experimental method including technology and validity of results
Unit 2 (L3)	Legislation, housing and risks
Unit 3 (M1)	Ethics and animal well-being
Unit 4 (M2)	Management of the practical experimental protocols & procedures

Table II: General content of the specialty divided in 4 units of 24 hours each (unit1, 2, 3 and 4) with the level corresponding in the course (L2 , L3 : second or third year of licence, M1, M2: first or second year of master

Undergraduate and postgraduate students' responses to mandatory courses (FELASA category C) in laboratory animal

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Summary

In 2003, FELASA established an accreditation system for courses adhering to the FELASA guidelines for the training of persons working with laboratory animals (categories A-D). At Uppsala University the FELASA category C course has been compulsory for students and staff planning and/or performing independent animal research since 1997. The present study scrutinized course evaluations from 524 students who attended the FELASA category C courses in laboratory animal science at Uppsala University from 1997 to 2003. The course evaluations demonstrated that the students realize that theoretical knowledge of laboratory animal science and practical skills are of great importance to the success of their future research involving animal experiments. All subjects and elements of the course, in particular the practicals on handling of conscious animals and procedures using live anaesthetized animals, were fully appreciated by the students. The FELASA C curriculum seems to be well received as a relevant introduction of laboratory animal science to young scientists prior to their independent work with laboratory animals in scientific projects.

Introduction

In order to improve and harmonise the training and education of persons working with laboratory animals in Europe, FELASA established an accreditation system for courses teaching the curricula of FELASA courses category A, B, C and D, in effect from January 1st 2004 (please see the paper regarding the FELASA accreditation published in these proceedings). At the Medical Faculty, Uppsala University, undergraduate and postgraduate students have been educated in laboratory animal science, according to the curriculum specified in the guidelines for FELASA category C, since 1997. The undergraduate students are mostly students attending the 4-year Biomedicine programme at the Medical Faculty. The main purpose of the Biomedicine programme is to educate future scientists, of whom many are expected to work with laboratory animals. The postgraduate students are PhD students primary at the faculties of Medicine, Pharmacy, and Technology and Science, using laboratory animals in their research. Within the Medical Faculty the FELASA category C curriculum (Wilson *et al.* 1995) is mandatory for undergraduate students attending the Biomedicine Programme and for PhD students who has an element of animal experimentation in their projects. The main object with the course is to give students the necessary knowledge and skills for performing independent animal research.

The course is given for 80 hours during two weeks, with lectures before noon and practical sessions in the afternoon. The main topics covered by lectures are biology and husbandry of laboratory animals; microbiology and disease; health hazards and safety practices in the animal house; design and conduct of animal experiments; anaesthesia, analgesia and experimental procedures; alternative methods; ethical aspects and legislation and finally critical analysis of scientific literature. The practical sessions consist of handling of small rodents, starting with exercises on dummy rats, followed by handling of conscious rats and mice; experimental procedures such as blood sampling and injections on anaesthetised rats and mice; handling of live rabbits, guinea pigs and chickens; animal behaviour studies and finally study visits. The course is concluded with a written examination. The allocation of time to the different topics differ somewhat between the undergraduate and postgraduate curriculum, mostly due to that some of the topics are included in other parts of the biomedical students' education. After finishing their studies, however, all students have completed the same curricula.

To maintain a high quality of an education, and to confirm that the aims and objectives of a course are fully understood by the students, it is of importance to investigate how the students evaluate and respond to the specific course. This can be achieved by analysing course evaluations, and the information can be used to further improve the curriculum. Under- and postgraduate students' responses to our FELASA category C course (autumn term 1997 to spring term 2000) have recently been evaluated (Carlsson *et al.* 2001). This investigation revealed that the students were very satisfied with the course and understood the necessity of being taught laboratory animal science. The FELASA C-course had a significant positive impact on the students' opinion on the importance of laboratory animal science. The curriculum of our FELASA category C course has only been slightly modified since the analysis by Carlsson *et al* (2001), and still conforms to the curriculum required for FELASA accreditation.

The aim of the present study was to examine written course evaluations from undergraduate and postgraduate students attending the FELASA category C course between autumn term 2000 to autumn term 2003, in order to investigate the impact of the FELASA category C curriculum on students' opinion on the relevance of laboratory animal science as a subject. The results were compared to those obtained in the previous study by Carlsson *et al* (2001). An additional objective was to investigate the students' opinion on the importance of different topics included in the FELASA category C curriculum, and to examine whether these opinions differ between undergraduate and postgraduate students.

Methods

All undergraduate and postgraduate students attending the Uppsala University FELASA category C course between autumn term 2000 and autumn term 2003 were asked to complete a course evaluation in combination with their written exam. The course evaluation was compulsory, and all evaluation forms were collected and processed anonymously. In total, 307 course evaluation forms were completed, of which 138 were from undergraduate students and 169 were from

postgraduate students.

The students were asked to answer four major questions. Firstly, they were asked to give their general opinion on the course. Secondly, they were asked to give their opinion (little, some or major) on the relevance of laboratory animal science for biomedical research, before and after the course. Thirdly, they were asked to mention what topic covered in the course they considered the most important, and finally, they were asked to mention what topics they wanted added to, or expanded in, the curriculum. Except from the question regarding the students' opinion on the relevance of laboratory animal science, all questions were open ended.

The likelihood-ratio Chi-square test was used to compare between groups in figures 1-3. P values less than 0.05 were considered significant.

Results

The students' general opinion on the FELASA C curriculum was very positive. The majority (>95%) among undergraduate as well as postgraduate students, found the course excellent or very satisfactory. Only a few individuals (approximately 2%) were disappointed with the course.

Before attending the course, five out of the 307 students (2%) considered laboratory animal science to be a subject of little relevance. After attending the course, no students at all expressed this opinion. Before the course, 89 students (29%), considered laboratory animal science a subject of some importance, but afterwards only 17 students (6%) were of this opinion. The number of students considering laboratory animal science a subject of great importance increased from 189 (58%) before to 271 (89%) after attending the course. 11% of the students did not have an opinion before attending the course. However, this proportion of students was significantly lower after the course (6%), which indicates that most students had a positive opinion after attending the FELASA C course. The results are presented graphically in figure 1, together with the results of the statistical analysis. No statistical differences were found in opinions between undergraduate and postgraduate students (figure 2). The results of the statistical analysis are presented in the figure legend. The results from the present investigation were compared to the previous study by Carlsson *et al* (2001). The opinions on the relevance of laboratory animal science of all 307 students in the present study were compared to the opinions expressed by 217 undergraduate and postgraduate students attending the course in the period from the autumn term 1997 to the spring term 2000. No statistical differences were observed between the two periods, except from the category no answer, that was significantly higher before and after the course in the period 2000-2003 (figure 3). The results of the statistical analysis are presented in the figure legend.

The majority of the undergraduates (89%) as well as of the postgraduates (95%) stated their opinion on which topic covered in the FELASA C curriculum they considered most important. The most frequently stated was practicals, i.e. handling and basic experimental procedures on live anaesthetised animals, which was mentioned by 42% of the undergraduate and 46% of the postgraduate students. Other topics that were considered most important were animal welfare; general knowledge in laboratory animal science; planning and design of animal experiments; laws and guidelines, and ethics (table 1).

33% of the undergraduate and 44% of the postgraduate students had suggestions on new topics to be added to the curriculum, or topics that should be expanded in the course. The suggestions were generally topics that should be expanded in the curriculum rather than new topics that should be added. In total, the students mentioned 28 different suggestions of topics that should be expanded in the curriculum. Many of these suggestions were, however, mentioned only by one or two students in either student group. The suggestions of topics to be added or extended are presented in table 2. Only topics mentioned by 5% or more of the students are presented.

Discussion

The present study demonstrates that the mandatory courses in laboratory animal science, following the FELASA category C curriculum given at Uppsala University, are generally well received by undergraduate and postgraduate students. The curriculum has a major impact on the students' opinion on the relevance of laboratory animal science. The results are in good agreement with those obtained in the previous study by Carlsson *et al* in 2001. Hence, the impact of the FELASA C curriculum on the students' opinion has not changed between the two periods 1997-2000 and 2000-2003. This is a good indication that the opinion depends on the content and organisation of the curriculum rather than on individual teachers, since there has been major changes to the teacher faculty during this time. We consider it satisfactory that the FELASA C curriculum has this great impact on the students. A high level of knowledge and manual skill among scientists is vital for upholding the welfare of the animals used as well as the quality of scientific results (Cohen 1966). Thus, it is evident that the FELASA category C curriculum is very appropriate for the students' understanding of the relevance of laboratory animal science for biomedical research.

The analysis clearly demonstrates that the practical sessions including handling and experimental procedures are considered very important by both undergraduate and postgraduate students. This observation supports the idea that practical training with handling of conscious animals and procedures on anaesthetised animals are important for achieving good skills in working with laboratory animals. Several studies have shown that hands-on laboratory activities add significantly to learning biology (Keiser and Hamm 1991, Mayer and Hinton 1990, Offner 1993). We consider that an important reason for the positive response of the students could be the way that the practical sessions are performed. No students are allowed to start handling or performing any procedures on the animals until they have been thoroughly instructed by a teacher, demonstrating how to pick up, restrain, anaesthetise and perform procedures on the animals. The teachers performing these demonstrations are well experienced in experimental procedures, and well aware of the importance of showing respect for the animals, as described by Hau (1999).

The other topics that were considered important among the students were generally mentioned in rather similar frequencies in both student categories. Animal welfare seems to be appreciated as an important topic among both undergraduate and postgraduate students, even though the undergraduates listed this topic a little more frequently than did the postgraduates. Planning and design of experiments were considered an important topic by 11% of the postgraduate students, but only by 3% of the undergraduate students. This topic is more relevant to the postgraduate students, since they are expected to play an active role in the design of experiments with laboratory animals. For the

undergraduates, on the other hand, the importance of this topic is probably not obvious at their stage of education.

The students' suggestions of new or expanded topics should be interpreted carefully, since only one third of the undergraduate, and less than half of the postgraduate students actually had any suggestions. In addition, many of the suggestions are likely to reflect specific interests by individual students. However, there are some interesting consistent recommendations. One of the most frequent suggestions in both student categories was that practicals sessions should be extended even further in the curriculum. This clearly indicates that the practical work with animals is well appreciated by the students, and that this is a very relevant topic for the students. Some postgraduate students suggested that species other than mice and rats should be included, probably because they used non-rodents in their individual projects. Some students, especially undergraduates, wanted more ethics covered in the curriculum, which is interesting, since rather few of the students considered ethics as the most important topic. However, this may be because this particular topic always is associated with good discussions with the students and is of more general interest than most other topics taught in the course. The postgraduates did neither consider ethics very important nor did they want the topic expanded. One explanation to this is that ethics in biomedical research is included in another mandatory course at the Medical Faculty, which teaches ethics to a much greater extent than what is included in the FELASA C curriculum. The fact that some of the undergraduate students wanted study visits to be expanded is because these have recently been restricted due to risk of infections in the animal facilities. All students making this suggestion were attending the course in question, and were also aware of the reason for the removal of study visits from the curriculum.

In conclusion, the FELASA C curriculum is well appreciated by the students, and has a major impact on the students' opinion on laboratory animal as a subject. All the subjects and elements of the course, in particular the practicals on handling of conscious animals and procedures using anaesthetised animals, were fully appreciated by the students. Thus, the FELASA category C course seems to be an appropriate and relevant introduction of young scientists prior to their independent work with laboratory animals in scientific projects.

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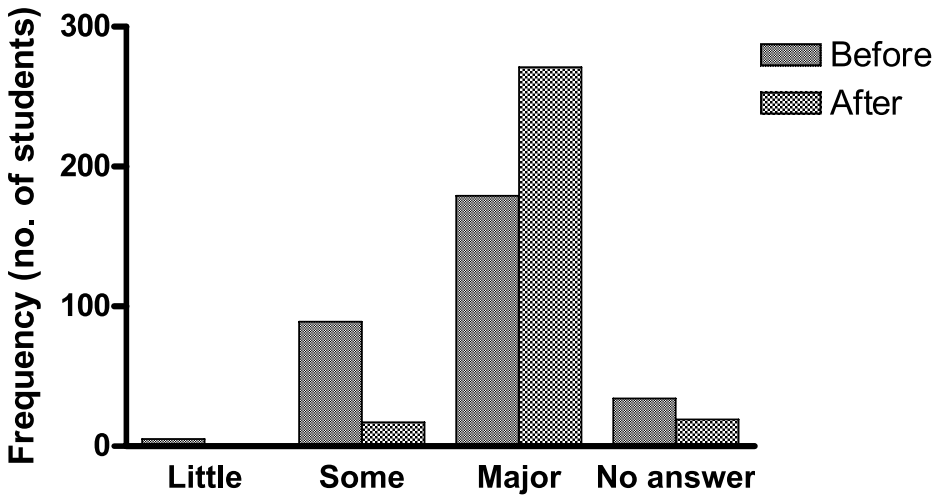

Figure 1. The opinion on the relevance of laboratory animal science as a subject before and after attending the course, of all 307 students attending the FELASA category C course between autumn term 2000 and autumn term 2003. The frequency is expressed as number of students stating each opinion. Statistically significant differences were found before and after the course for all opinions. The likelihood ratio was 6.97 ($p < 0.01$), 63.844 ($p < 0.0001$), 73.67 ($p < 0.0001$) and 4.71 ($p = 0.03$) for the opinions little, some, great and no answer respectively.

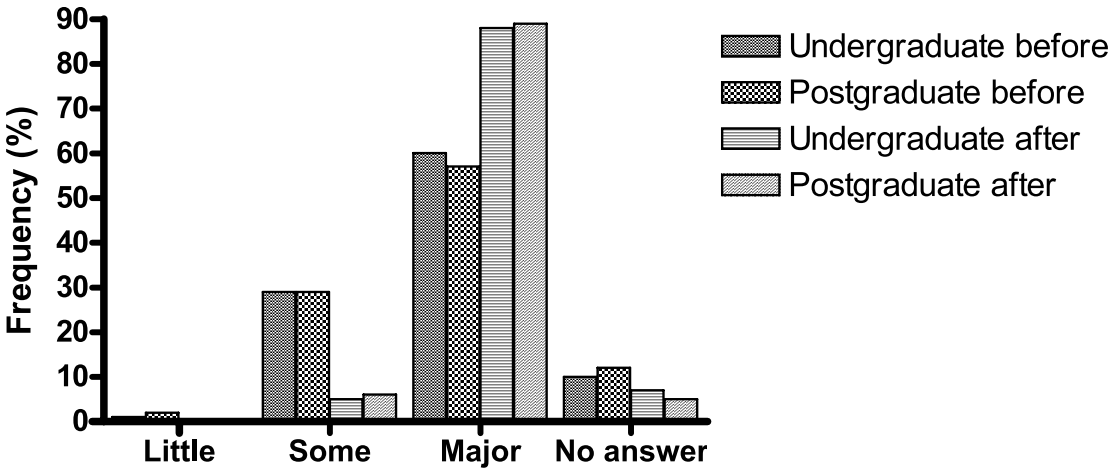

Figure 2. The opinion on the relevance of laboratory animal science as a subject before and after attending the course, of undergraduate students in comparison with postgraduate students between autumn term 2000 and autumn term 2003. The frequency is expressed in percent of the total number of students in each category. No statistical differences between the two student categories were found in any of the opinions. Likelihood ratio d" 0.36, p e" 0.55.

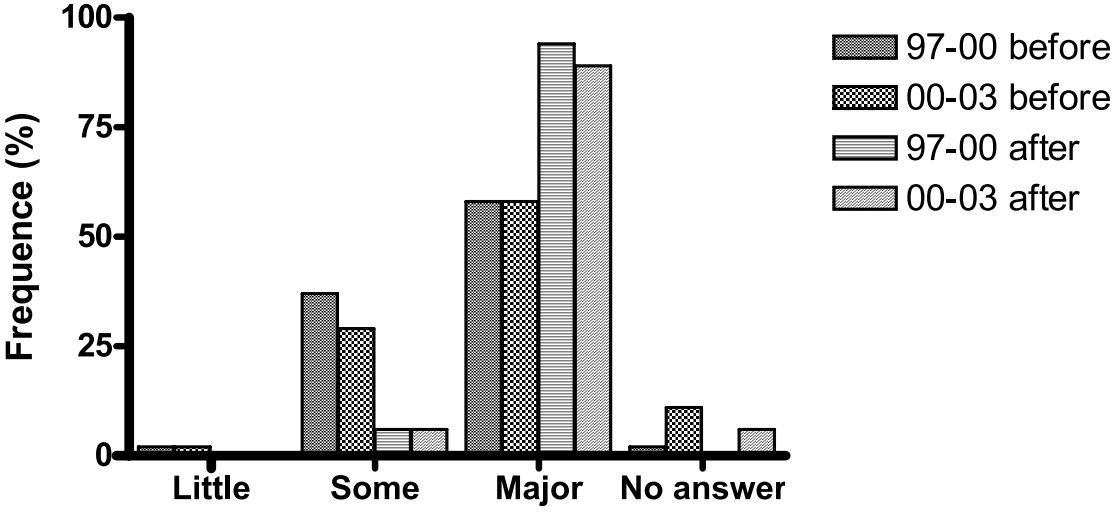

Figure 3. The opinion on the relevance of laboratory animal science as a subject before and after attending the course, of undergraduate and postgraduate students attending the course between autumn term 1997 to spring term 2000 (217 students), in comparison to those attending the course between autumn term 2000 and autumn term 2003 (307 students). No statistical differences between the two periods were found in any of the opinions (likelihood ratio d".1.63, p e" 0.20), except from no answer that was significantly higher before and after the course in the period 2000-2003. The likelihood ratio was 7.29 ($p < 0.01$) and 8.50 ($p < 0.01$) before and after the course respectively.

Table 1. Most important topic covered in course

	Undergraduate students n = 123 [89%]		Graduate students n = 160 [95%]	
Practicals	52	(42 %)	73	(46 %)
Animal welfare	37	(30 %)	37	(23 %)
LAS in general	31	(26 %)	28	(18 %)
Planning/design of experiments	4	(3 %)	18	(11 %)
Laws and guidelines	13	(12 %)	12	(8 %)
Ethics	4	(3 %)	11	(7 %)

Table 1and 2: Most frequent opinions on the most important topic covered in the course of undergraduate and postgraduate students respectively. The percentage expressed within parenthesises is the proportion of those students that answered the question. Only topics mentioned by 5% or more in either category are included in the table.

Table 2. Topics to be added or expanded in course

	Undergraduate students n = 46 [33%]		Graduate students n = 75 [44%]	
Practicals	7	(15 %)	16	(21 %)
Species other than mice and rats	1	(2 %)	11	(15 %)
Comparative biology	6	(13 %)	10	(13 %)
Ethics	8	(17 %)	8	(11 %)
Experimental methods	7	(15 %)	7	(9 %)
Transgenic animals	3	(7 %)	6	(8 %)
Anaesthesia and analgesia	1	(2 %)	6	(8 %)
Lab animal behaviour	1	(2 %)	6	(8 %)
Handling of larger animals	0		5	(7 %)
Laws and guidelines	0		4	(5 %)
Study visits	8	(17 %)	2	(3 %)
When to use lab animals	3	(7 %)	2	(3 %)
Planning/design of experiments	5	(11 %)	0	

EURCA – a co-operative European project to support the use of non-animal undergraduate education.

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Summary

There are a large number of non-animal models available to support teaching. The choice of which model to use depends on tutors clearly defining the learning goals. There are several on-line databases to help teachers in this choice. One of these is the EURCA database (http://www.eurca.org), which offers extensive information on high-quality peer-reviewed models. In an effort to increase awareness, EURCA also gives demonstrations of several models and provides advice to teachers at national and international meetings. Currently, EURCA has accomplished a network of national contacts in each EU country.

Animals are still being used in practical classes in pharmacology, physiology, laboratory animal sciences, anatomy and dissection classes. These classes are particularly useful when learning practical skills are part of the objectives. However, undergraduate labs, in particular, are also used for teaching factual knowledge, and skills such as data handling, experimental design, and communication. They have some disadvantages, they are resource intensive drawing heavily on student and teachers time, require technical support, equipment, consumables, animals, and specialist accommodation and students may have negative perceptions if their experiments fail. Non-animal models can be a less expensive alternative for teaching knowledge and many of these skills. Most models are computer-based simulations, but static and interactive video, post-mortem material and in vitro methods are also being used. They have several advantages, they are less expensive and several studies have demonstrated that knowledge gain is equivalent to animal classes and data handling skills, experimental design skills and communication skills (oral, written) can be effectively taught. Many include features, which make them suitable for study independent of tutor support e.g. inbuilt on-screen support, self-assessments and a generally good combination of theoretical and practical components. Furthermore, by using these models instead of animals, a contribution is made to the reduction of unnecessary animal use.

It is current EU policy to replace, reduce and refine animal experimentation. EU directive 86/609 EEC (Article 7, 2) specifically states: 'An experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practicably available'. In addition, 'The European Convention for the protection of vertebrate animals used for experimental and other scientific purposes' (ETC 123: Article 25, 3) states, when referring to animal experiments in education: 'Procedures referred to in paragraph 1 of this article shall be restricted to those absolutely necessary for the purpose of the education or training concerned and be permitted only if their objective cannot be achieved by comparably effective audio-visual or any other suitable methods'.

Central to this is the promotion of ethical awareness and attitude building in young scientists to create an environment for young scientists in which new approaches to research will be fostered. A key aspect of this is to replace unnecessary animal use in education where, across Europe, there are still significant numbers of animals used for teaching. The use of good quality non-animal models in higher education will contribute to the development of a student's attitude towards experimental animals. Over recent years there have been significant developments in alternatives to animal experiments in education and there is now enormous potential to have a real impact on the number of animals used in education. There are strong ethical reasons for the use of alternatives and teachers, scientists, and society at large recognise a moral obligation to reduce animal suffering wherever possible. There is also considerable evidence to support the view that using alternatives represents good educational practice and that alternatives are often less expensive than using animals. To realise a reduction in animal use it is important to target and influence teachers in higher education who 'drive the educational process'. In particular they need to be made aware of the existence of alternatives, provided with evidence of their usefulness and given advice about how to integrate them into their teaching practice.

EURCA

In an effort to address this, the EURCA (European resource Centre for Alternatives in higher education) project was initiated in 2001. This project was co-directed by the Universities of Edinburgh and Utrecht. The activities of EURCA are in line with and fully support European legislation and are based on the recommendations made in an international workshop on alternatives in education.1

In its first three years, the EURCA project has focussed on the following:

Outreach activities:

- taking the 'alternatives collection' to major international scientific meetings to act as a one-stop advice centre for teachers and to provide the opportunity for teachers to try-out alternatives to assess their usefulness;
- raising the profile of alternatives by presenting papers/ posters at international meetings about the use of alternatives.

Dissemination activities:

These include:

- the establishment of an internet website (www.EURCA.org) comprising: an information-rich database of alternative resources; evaluations, independent reviews, links to users, information, discussion forum, news and

events, bulletin board, producing a quarterly newsletter which is widely circulated.

The web site is the entry point to EURCA for most teachers.

These are all ongoing activities and most of the project's resources are directed towards the continuation of this service.

Despite the above-mentioned activities, there have been difficulties in reaching some teachers who are unaware of the existence of EURCA and the pedagogical potential of alternatives. There has also been some resistance to using some alternatives, as many are only available in the language of the country in which they were developed. Furthermore, existing alternatives may not fully meet the learning goals of a particular learning assignment.

New initiatives

To facilitate communication between EURCA and teachers in each EU country, activities were undertaken to build a network of national EURCA coordinators. Currently there are 21 active national co-ordinators/project partners from European countries. Each of the national partners of this project is represented by a senior academic working in a major university whose role is to co-ordinate a national network of academic colleagues from other universities in their own country. Thus, each national contact potentially represents a large number of institutions in their country.

There are two primary partner institutions involved in the EURCA network. The network is jointly managed by the Utrecht University (UU) and the University of Edinburgh (UE).

The network will operate on a hub and spokes model. The hub (EURCA at Edinburgh and Utrecht) will co-ordinate and manage central outreach and dissemination activities. The hub will direct the activities of and provides support to the network of national contacts who have specific, agreed responsibilities in their own countries.

The national contact in each country will co-ordinate a local network of academic teachers working in institutions (universities) in that country in which there is animal use for teaching. The number of institutions will vary from country to country but typically would be approximately 10-20 per country. These local networks will be the spokes of the system. National contacts are brought together each year at an annual meeting to facilitate information exchange, sharing of ideas and reports on activities.

Furthermore, it is anticipated that in the near future, initiatives will start to have alternatives translated in the national language.

Target groups

Target groups for EURCA are teachers in biomedical (e.g. pharmacology, physiology) and veterinary sciences. These are the drivers of the educational process who will ultimately affect how teaching and learning are delivered, determining whether animal labs are used or not as part of the educational process and thereby influencing the attitude of students towards animals.

Since the activities of EURCA are widely published and disseminated it is also anticipated that students and interested members of society will be involved and encouraged to contribute to the exchange of information and discussions.

The open access databases gives excellent opportunities to disseminate the outputs of EURCA. In addition, visits to national and international meetings by EURCA partners who will demonstrate alternative models and provide information about EURCA activities will further the ultimate goal of the project to aid teachers to apply the best practice educational models to their teaching thereby reducing animal experiments.

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Development of a career ladder for laboratory animal technicians in the United States of America

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Summary

The American Association for Laboratory Animal Science (AALAS) has developed a career ladder for laboratory animal technicians in the United States of America (USA), and the program is available for AALAS member technicians (you don't have to be an AALAS member to take the exams – it costs more) throughout the world. Personal growth and other educational points are discussed. The AALAS program includes a Technician Certification Program containing three levels of certification, a three level Registry, availability of an internet learning library (ALL), a two-year program entitled the Institute for Laboratory Animal Management (ILAM), and a professional certification program for Laboratory Animal Resource Managers (CMAR).

The American Association for Laboratory Animal Science (AALAS) has developed a career ladder for laboratory animal technicians. We have many international members that have become certified – especially in Canada. The demand for fully trained, competent laboratory animal care technicians in the field of laboratory animal science has greatly increased as the need for medical, technical and experimental scientific advances arise.

Beginnings

As one starts building a career within the field of laboratory animal science it is important to start with as much formal education as possible. My own career started with a degree in Medical Technology with Bacteriology as my specialty. However, the AALAS program is intended to be inclusive and does not require a formal education beyond High School or GED.

In addition to formal education it is important to maintain a positive caring attitude and being of an inquisitive mind regarding science and the animals in ones charge. For example my first encounter working in a mouse breeding facility introduced me to differences in animal behavior and breeding habits of the various mouse strains maintained within the facility. Take advantage of learning opportunities as they present themselves within local or national programs available. Be active and build a network with other technicians. Set a clear course for continued education.

AALAS Technician Certification Program

The AALAS certification is the highest recognition for technicians in the laboratory animal science profession in the USA, and it is recognized on a world-wide basis, including *The Guide for the Care and Use of Laboratory Animals*. The program was developed to recognize professional achievement and provide an authoritative endorsement of a technician's level of competence in laboratory animal technology. Resource kits are available on each certification level with appropriate workbooks, manuals, and CD-ROMs in English and in Spanish. A workbook is available for the student, a teacher handbook, and a teacher desktop version on each level of certification. A free Technician Certification Handbook is available explaining the requirements for each level from AALAS. Additionally, there are courses for each level in the on-line, AALAS Learning Library for individual and group education.

Laboratory animal technicians are certified at three different levels of competency. Certification at each level requires meeting prerequisite education and experience and passing a corresponding certification examination.

The entry level is the Assistant Laboratory Animal Technician (ALAT) which requires one year employment in a laboratory animal facility. The second level is the Laboratory Animal Technician (LAT) which requires at least three years employment or a combination of appropriate college education and experience totaling 3 years. The third and highest level is the Laboratory Animal Technologist (LATG) which requires five years employment or a combination of formal education and employment.

One does not have to certify on all three levels in succession. For example, in my case, I certified as an LATG without having to take the ALAT or the LAT certification examination before.

AALAS Technician Certification Registry

Participation in the technician certification Registry is a visible distinction of personal professional achievement and dedication. The Registry recognizes technicians who choose to maintain high educational standards and display a current credible level of knowledge. Registry members are distinguished by an "R" preceding their certification level acronym: RALAT, RLAT, and RLATG.

To maintain Registry status, participants must earn a minimum number of Continued Education Units (CEU's) every two years, depending on the certification level. Participation in the Registry demonstrates caring, initiative, and responsibility that are all superb characteristics of a laboratory animal technician. There are no annual fees to belong to the Registry.

It is important to keep informed about new techniques and to understand the reasons of the development behind the techniques. Utilize your knowledge and form alliances with other groups of technicians for mutual benefit. Take advantage of available programs and expand your horizons. The Registry is a demonstration to current and future employers that one has maintained current knowledge in the field.

AALAS Learning Library (ALL)

In July of 2003 AALAS opened the electronic doors of ALL. The program features courses, exams, transcripts, continued education units (CEU's). ALL offers a unique opportunity to access web courses with exams including group management with documentation for training

coordinators. The courses are designed to be of benefit to technicians, managers, veterinarians, researchers, and members of Institutional Animal Care and Use Committees (IACUC). Additional courses are added as they are needed and developed.

Examples of courses included are "Post Procedure Care of Rats and Mice"; courses based on the AALAS Training Manuals for technician certification; and a series of courses preparing investigators to work with an IACUC. New online courses such as "Ethical Decision – Making in Animal Research" with three companion courses that includes case studies are available on ALL since early 2004.The ALL program offers specific educational courses and can therefore be individualized as to the need of the person taking the courses.

AALAS Annual Conventions

Annual visits to the AALAS Convention is a definite must for technicians and is another way of keeping up-to-date with new information concerning different species, techniques, and developments in laboratory animal science. One can find a multitude of platform sessions, seminars, poster sessions, and special topic sessions to attend. Another source of information is offered at the Learning Resources/Technology Center. Over 150 videos are available on a multitude of subjects such as "Handling/Care/Biomethodology" of a variety of laboratory animal species; "Veterinary Medicine/Lab Animal Medicine"; and "Basic Surgical and Anesthetic Techniques/Skills". The national meeting also hosts the largest number of exhibitors in the industry, which is a unique way to be updated on new and traditional products and services.

AALAS Institute for Laboratory Animal Management (ILAM)

ILAM is an AALAS educational program developed to provide instruction in management concepts that is applicable to the laboratory animal science industry and to enhance communication, team building, and networking among colleagues with mutual interests. The ILAM program began in 1992 and the first class graduated in 1993.As directors, managers, and supervisors of laboratory animal facilities see their roles grow more and more complex over the years. Individuals in management positions must be able to interpret the social, political, and economic environments in which they operate.

The program includes 64 class-room hours instruction over a two-year program. The school provides a progressive program which requires 32 hours of instruction annually. Class topics vary from year to year depending of the needs of the industry and upon the request of the students. My own personal experience attending ILAM, as part of the inaugural class, became a most important part of my own career building. My networking capabilities and friendships stem from that experience.

AALAS Certified Manager of Animal Resources (CMAR)

In 1999 AALAS and the Laboratory Animal Management Association (LAMA) partnered with the Institute of Certified Professional Managers (ICPM) to establish a certification program designed specifically for the laboratory animal resources manager. The first Animal Resources examination was given at the AALAS Convention in Baltimore, Maryland in October of 2001.

The eligibility requirements are as follows:

- A Bachelor (BA or BS) or higher degree from an accredited college or university.
- Five years of laboratory animal-specific experience.
- Three years of management or supervisory experience in the laboratory animal field. Management or supervisory experience should include hiring, firing, and/or direct supervision of employees; administering performance appraisals; managing facility resources; and/or managing a budget.

The CMAR program includes three "Certified Manager Examinations" which are administered by ICPM. The candidates are tested for 1) Personal Skills; 2) Administrative Skills; and 3) Interpersonal Skills. The fourth module contains the Animal Resources Examination.

The additional certification as CMAR provides the certificate holder with recognition as an industry professional and employers often rely on certification credential as a factor in promotion or hiring decisions. The certification process helps the manager to evaluate strengths and weaknesses. Having achieved the CMAR will build the individuals confidence and improves performance in a positive manner.

Conclusion

The laboratory animal technician in the United States can, through their own interests in combination with all programs offered by AALAS, become a valuable asset to the laboratory animal science profession.

My own stepping stones, when building my career ladder, stems from attaining certification and maintaining subsequent registry, attending AALAS annual conventions, attending ILAM, and by being aware of changes in the laboratory animal science industry. The technician can build a career ladder taking them from a humble beginning as an Assistant Laboratory Animal Technician up to a Certified Animal Resources Manager. AALAS and the technicians own personal drive can make it possible.

Enrichment, Welfare and Animal Housing

Happy animals make good science: when does environmental enrichment make animals happy?

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'Happy animals make good science' was once stressed by Trevor Poole (1997, Lab. Anim. 31: 116-124). Environmental enrichment (EE) is considered as a tool to make animals happier. However, concerns have been voiced that EE might increase variability and therefore may require more animals for the same results. Furthermore, it has been reported that introduction of EE without proper planning and forethought may be useless or even harmful to the animals. A FELASA working group on standardization of EE has been appointed to address these concerns. The presentation will discuss which EE procedures are common and how they may contribute to a better life of laboratory animals by fulfillment of their environmental needs, improved stress coping, and fear reduction. Concerns regarding EE-induced higher variability will be weighed against possible gains in validity of the animal models. It is concluded that before EE procedures are introduced on a broad scale, they need to be evaluated by scientists specialized in this area. Furthermore, EE procedures need to be described in the methods section of publications and reports to facilitate the reproducibility of experimental conditions. Just as with 'hardcore' laboratory animal science three to four decades ago (i.e. standardization of climate, health status and nutrition), the introduction of EE will be a continuous process. It is part of the refinement component within the 3R-concept, needs to be based on sound scientific principles and has to take into account practical aspects as well.

Preliminary evaluation of basic enrichment in various breeding conditions with different rodent species (mice, rats and guinea-pigs) and strains: practical consequences for the implementation of an enrichment programme in breeding facilities.

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The current revision of Convention ETS 123 (4th. Multilateral Consultation) is recommending significant evolutions in the field of caging space and density, harmonious social groups and environmental enrichment. It is also advising against producing invalid data, the ability to inspect animals with minimum disturbance, to handle them easily and frequently and to avoid any waste of animal lives.

It should also be possible toguarantee reliable bio-security practices (control of the contamination risk and the health quality), the stability of biological data and hence the quality of research.

Optimisation of suitable environmental conditions and of an enrichment programme, both for animal welfare and scientific purposes, is dependant on the research application and generally well-defined expectations and specifications. It can be easily designed on a case-by-case basis.

In contrast, animals in large commercial breeding colonies are generally used for multiple research uses, each with specific requirements or limitations, which are not always compatible e.g. neurobiology, behaviour studies, toxicology, metabolism and bioassays. Another basic user requirement is for a fixed and standard breeding environment to comply with GLP, audits and delivery agreements.

One concern is a variation of breeding environmental factors that may influence the characteristics of the animal model or the consistency and stability of breeding conditions over time, biosecurity, efficient observation / inspection of the animals with minimum disturbance, influence on phenotypic expression (variability, stability)…

In consequence, all these issues should be very carefully addressed when designing and assessing an enrichment programme. Furthermore it is also critical to evaluate the benefits and drawbacks for each strain or stock, disease model, sex / age category, breeding and caging system... and in some cases to consider the potential interaction of refinement with another "R": reduction.

It is now currently accepted that, depending on its quality and relevance, an enrichment programme can be as harmful as beneficial and generate both a negative and positive impact on research, directly or indirectly. For example, an inadequate design or position of a "shelter" in a rodent cage can block or limit the access to the water bottle.

Since the 1960s, numerous publications have reported that enrichment influences learning, memory, synaptic capacity and can even compensate for genetical deficits. It is not only true for the experimental environment (directly controlled by the investigator) but also for the breeding environment. One can only advise to visit the breeding facilities and to review with the breeders the key housing and caging conditions potentially influencing the characteristics of the animal models.

In another field, drug safety evaluation, several enrichment approaches can potentially interfere with or invalidate an assay, others may increase variability or impair data analysis. These include diet & nutrition (ingredients and quality, formula, distribution);

inert or inedible devices (stainless steel, some plastics); edible items (bedding, wood sticks, nesting materials, plastics); toys, shelters & platforms; and cage design & effects (social groups *vs* individual caging).

In order to pave the way to future applications, our Ethical Committee appointed a working group in order to carry out a series of preliminary evaluation with simple enrichment approaches. Assessing the use of various types of shelters, we could observe a decreased use of the cage surface, in favour of an increased time in the very limited space under the shelter. Rats and mice are nocturnal species and keep use the shelters at night as shown in the following table (after *W.J. White*).

In parallel to this decrease of utilization of the available space, a decrease of activity was also observed, in particular with C57BL/6J mice. In the same strain, 6 week-old females kept in type S (424 cm^2) filter-top cages barely used the shelter, when placed perpendicular to the cage length. After a 90° rotation, they started staying under the same tunnel most of the time!

Other preliminary evaluations were conducted in ML open cages (870 cm^2) equipped with a stainless steel tunnel, with various mouse strains and stocks. Mice were observed between 21 and 35 days of age (with a cage of density = 22, male & female cages).

2 x 2 feet cage, 20 rats 200 to 300 g
% of total surface occupied (AM, before noon, PM afternoon)

Without shelter	39% AM	27% PM
With shelter (~25% cage surface)	12% AM	17% PM

They showed:

- A "time effect": use of clean and dirty areas during the first week, then almost no difference of space utilisation during the second week.
- A "strain effect":
  . BALB/cByJ: « in / out » movements but no stay under shelter, males and females behaving in a similar way, with a less aggressive male behaviour (bites);
  . C3H/J: females behaving as BALB/cByJ but males blocking the tunnel openings with bedding, before getting disinterested and moving over the shelter.
- A "sex growth effect" in BALB/cByJ, over the observation period:
  . Female growth: + 4.6 g (no shelter) and + 5.5 g (with shelter)
  . Male growth: + 6.2 g (no shelter) and + 6.4 g (with shelter)
- A "cage density effect" (after experimental density increase of + 50 %):
  . Female growth: weight increase to 5.4 g (no shelter) and 6.4 (with shelter)
  . Male growth: weight decrease to 4.1 g (no shelter) and 4.5 (with shelter)

With C57BL/6J and other strains, the most interesting results were obtained with bedding & nesting material such as wood shavings or cotton. However, a very significant drawback of cotton was the need to disturb animals' nests for visual inspection, with in extreme cases pups "trapped" in a "cotton" web, complicating their handling.

With rats (OFA-SD females, 6 week-old, 3 per cage in type III cages / 803 cm^2) we compared different types of bedding with & without shelter. The main observation was an almost permanent stay under the shelter, where the space got overcrowded. The food intake was found unchanged but the water intake increased by 100 ml over one week.

In some case, and unexpectedly, a minor change generated a huge benefit. With group-housed guinea-pigs, kept on bedding (saw dust type), a simple plastic ring hanging on the cage side seemed to provide some distraction and dramatically improved the ear lesions due to biting, decreasing the percentage of incidence from more than 10 % to nothing! Unfortunately, after a few weeks, the animals appeared to get used to this simple device, and the biting behaviour increased (to a lower level), so other complementary or alternative enrichment approaches will have to be used.

In summary and as a first conclusion of these preliminary evaluations, for any potential enrichment approach, we decided to balance carefully the combined benefits and drawbacks both for animal welfare and good science and to duly consider not only the individual benefit of enrichment but also the group benefit represented by the total number of animals to be used for the same experimental result (i.e. the increase or decrease of units per group to reach the same statistical conclusion). In other words, it means weighing the "refinement" benefit versus the "reduction" benefit.

Breeding multi-purpose and highly standardised research models, requires strict control of biosecurity & contamination risk and assessment of any enrichment with all stocks and strains, including all ages and both sexes over time. This cannot be done by the breeder alone but requires a close collaboration with representatives of the different categories of users. As with any major modification of the breeding conditions, it should be clearly documented and made available for the investigators.

With any enrichment system, its positive impact such as reduction of aggressive behaviour, biting, hair loss, reproduction of sensitive models, expression of normal behaviour pattern, etc. should be carefully balanced with the potential drawbacks such as an increase in experimental variability, stability of biological data, disturbance & increased aggressiveness and decreased quality of inspection. Collecting observations over an extended period of time is also. Even if the current revision of ETS 123 stresses enrichment, Directive 86/609/EC article 7 also requires the selection of different protocol designs aiming at the same objective, the use of the "lowest number of animals".

Volatile organic compounds in animal bedding and enrichment items

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Bedding volatile compounds were shown to induce liver microsomal enzymes in mice 35 years ago. This effect was due to presence of high concentrations of pinenes in the bedding, a finding verified several times thereafter. Survey of commonly used rodent beddings five years ago showed that that some beddings were still loaded with pinenes and eight other arbitrarily chosen compounds, and that autoclaving made most of the compounds disappear. More recently a vast variety of enrichment items, meant for use inside the cage, have been introduced. Some of them are made of same materials as bedding or some other organic material. As such, they pose a similar source of chemical interference to research as bedding. This study was designed to screen commonly used beddings and enrichment items for detectable volatile organic compounds. Fifteen bedding and 16 enrichment item samples were collected from the manufacturers. Volatile organic compounds were determined by using a Chrompack M 16234-89-1 purge and trap injector connected to a Hewlett-Packard 5891II gas chromatograph with a Hewlett-Packard 5971 mass selective detector. The gas chromatograph was equipped with a JScientific fused silica capillary column (DB-VRX, 30 m, 0.25 mm, 1.4 _m) and quantification was performed by a total ion recording method using 1,3,5-trichlorobenzene as an external standard. The following 20 compounds were identified (present in number of bedding/enrichment samples): propanal (2/0), pentanal (9/4), hexanal (16/11), heptanal (6/5), octanal (4/4), nonanal (15/6), toluene (8/3), 2hexanone (6/0), a-pinene (3/1), b-pinene (3/0), 3-carene (2/0), limonene (1/1), a-phellandrene (0/1), b- phellandrene (0/1), b-myrcene (0/1), 1,3,8-p-menthatriene (0/1), caryophyllene (0/1), 1-okten-3-ol (1/0), 2-heptenal (1/0), 2-oktenal (1/0). Concentrations of the volatile compounds were highly variable, ranging up to 550 ng/g. Only a few samples contained pinenes indicative of softwood origin. Some manufacturers had enrichment items made of the same material as their bedding, and the results showed that this approach did not introduce new volatile compounds into the cage. In conclusion, use of enrichment items of organic origin other than that used in bedding, increases chemical burden in the cage, and hence may be a potential cause of interference to the study.

The use of IVC-systems for housing mice and rats

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Summary

Individually ventilated cage systems (IVCs) are commonly used for housing mice. It began in the 1950s with the development of the first filter top cages, and since then the development has resulted in the filter top cages known today with several different designs and cages being available. The number of air changes varies from 30 to 120 per hour. To achieve this rate, air must enter the cage at high speed, but in most systems the air streams are regulated and at animal level most systems have air speeds below 0.2 m/s. When rodents are housed in IVC-cages, they may be affected by the number of air changes and the air speed (draught) in ventilated cages and the level of CO_2 in unventilated cages. Studies on different ventilation conditions do not indicate that the level of air speed affects rats and mice, whereas a high number of air changes (above 50-60 per hour) are avoided by the animals if possible. CO_2-levels above 3% seem to affect the animals and are avoided by them. The IVC-cages are not to be left without ventilation for long periods, as the level of CO_2 may reach 3% within 20 minutes depending on the air-tightness of the cage, which again seems dependent of the brand.

The history of the IVC-systems

In the 1950s Dr. Lisbeth Kraft was carrying out research on rotaviruses, the cause of epidemic diarrhoea in mice (Kraft 1958). To prevent the spread of the virus to the surroundings, she developed a metal cylinder with wire mesh walls wrapped in fibreglass insulation and with a metal top and bottom. Inside the cylinder, bedding, water and food for the mice were placed.

This cage effectively protected the environment against the virus, as well as the mice against other infections. In the next decade the filter top was further developed to fit a normal shoebox cage still using fibreglass insulation as filter medium.

The filter top became a success in research, and had few disadvantages. Most animal facilities installed various technological equipment for regulation of temperature, humidity and air quality, still leaving environment inside the filter top cage uncontrolled. In the early 1970s the first measurements on the microenvironment inside the filter top cages were conducted (Serrano 1971, Murakami 1971, Simmons et al. 1968) revealing that the filter top had major effects on intra-cage temperature, humidity and trace gases such as CO_2 and NH_3. The temperature inside the cage was 1-2 °C higher than the surroundings, and the relative humidity 10-15% higher than that of the room. Compared to an open cage the CO_2-level was ten times higher in a cage with filter top, and during the active period reaching up to 0.8 %, and NH_3 levels up to 400 ppm were measured inside the cage. In spite of these effects on the cage environment the static filter top is still in use today, but in a more practical design. In 1980 Robert Sedlacek invented a new type of static filter top fitting the shoebox cage with a more practical filter media compared to the fibreglass insulation. The filter top was placed on top of the cage with an overhang along the cage edge, like the principle used in the petri dish. Most static filter tops today are based on that design.

For improvement of the environment inside the filter top cage, each cage can be ventilated with clean fresh air. Although there is spontaneous air change between the cage and the environment, this may not be enough to secure the air quality inside the cage. In the 1980s the first system equipped with individually ventilated filter top cages became commercially available, and in 1985 the word "microisolator" was accepted as a common name for a filter top cage. The term "microisolator" is derived from the traditional flexible film isolator. The cage is regarded as a closed unit comparable to an isolator, which is obviously not the case, as the cage never is as safe as the isolator. Nevertheless, the term is used for systems protecting the animals against infections from the outside (Baer et al. 1997). A microisolator do not have to be ventilated, but must be a closed unit, e.g. with a filter top, in order to protect the animals against infections (Hasenau et al. 1993, Keller et al. 1989). Today, IVC-systems are widely used for protection of animals and/or staff in the animal facility. Here only IVC-systems using filter top cages will be discussed, although there are other IVC-systems on the market using other barrier principles beside the filter top for protection of animals, e.g. MADU (mass air displacement unit), PIV (pressurized individually ventilated) cages, ventilated cabinets etc.

For the IVC-systems using filter top cages three different ventilation principles are used (Figure 2). The first type of ventilated filter top cage is equipped with one ventilator blowing air into the cage diffusing it out through the filter top (2A). This type of cage has an inside pressure positive to the room offering high protection to the animals inside the cage. Several experiments have shown that a cage running in positive mode is able to protect the animals against infections (Clough et al. 1995, Lipman et al. 1993, McGarrity & Coriell 1973, Mrozek et al. 1994). When the cage is ventilated the microclimate is improved considerably as gases such as CO_2 and NH_3 are removed efficiently and the bedding is dried reducing the growth of NH_3 producing bacteria (Corning & Lipman 1992, Huerkamp & Lehner 1994, Keller et al. 1989, Lipman et al. 1992, Perkins & Lipman 1996). In the 1990s laboratory animal allergy (LAA) and reduction of allergens in the room became an important issue. Setting the pressure in the cage negative to the room tends to keep the allergens inside the cage (2B). A ventilator drawing air out of the cage and air diffusing in through the filter top result in a negative pressure. This keeps the allergens inside the cage and prevents infectious agents spreading from infected animals. With the cage kept on negative pressure the release of allergens is reduced significantly (Renström et al. 2001, Sakaguchi et al.

1990). Today most systems are using two ventilators, an in-going and an out-going (2C). Normally, one of the ventilators is the one working harder, creating either a small positive pressure or a small negative pressure. This means that only a small amount of air is diffusing either in or out through the filter top. Using a system with two ventilators reduces the amount of air diffusing directly into or out of the cage by 70-80 percent depending on the settings of the ventilators.

For keeping the animals protected against infections, the inlet air must be passed through a HEPA-filter cleaning the air from infectious agents and particles with an efficiency of up to 99.97% (Mrozek et al. 1994). Also, to prevent spread of allergens from the animals to the room, the exhaust air can be ventilated through a HEPA-filter or ducted directly out of the room.

The impact of IVC-systems on animals

According to the vendors, up to 20% of European facilities may house rodents in IVC systems today. Especially for transgenic rodents the IVC-systems are considered useful as supplementary protection, but IVC-systems are also used to reduce release of allergens, and thereby preventing LAA among the staff (Renström et al .2001). There may be both advantages and disadvantages using IVC-systems. In recent years it has been disputed to what extent welfare is affected when animals are housed in IVC-systems. The question of concern when housing rodents in IVC-systems is how the animals are affected by a high air speed (draught) and a high number of air changes, as well as to which extent the animals are affected by levels of CO_2, when the cages are unventilated due to cage changing, transport of cages etc. (Krohn 2001).

Studies on rats have shown that the air speed (draught) probably is not of importance, as the animal seem unaffected by air speeds above 0.5 m/s (Krohn et al 2003b). For mice the picture is less clear. One study indicates, that the mice are affected by draught to some extend (Baumans et al. 2002), as the mice prefer cages without ventilation, although it is unclear whether the impact on the mice was caused by the draught specifically or the ventilation in general, as the two parameters were not separated. In another study, in which only the effects of draught were evaluated, the results are contradictory between preference and telemetric studies, so further analysis is needed before a conclusion can be drawn (Krohn et al. 2004).

Rats are affected by air changes above 80 per hour (Krohn et al. 2003b) and prefer cages with air changes below 80 per hour. If exposed to air changes above this, their heart rate and systolic blood pressure are increased, which is an indication of stress as previously shown (Krohn et al. 2003a). A high number of air changes seem to affect mice, as they prefer cages with air changes lower than 100 per hour (Baumans et al. 2002), although it is difficult to say whether it is the number of air changes or the draught that affects the mice. Another ongoing study seems to support the fact that mice are affected by a high number of air changes, although the results are not clear at the moment (Krohn et al. 2004). So, for mice, more studies are needed before a final conclusion can be drawn.

As a rule of thumb, when housing rodents in IVC-systems, the number of air changes should be fixed at 50-60 per hour, which is sufficient to ensure proper ventilation. The air inlet nozzle should be placed in the top of the cage, and as far away from the bedding as possible, to ensure that the animals are not exposed to draught. Finally, to ensure that the animals are able to find a place without any draught, they should be given some kind of bedding material for nest building, as that is a way to eliminate effects of high air speeds (Baumans et al. 2002).

Another issue affecting rodents in IVC-systems is increased CO_2 concentrations if the cages are not properly ventilated. CO_2 is not directly lethal to the animals (in lower concentrations), but studies have shown, that exposure to levels above 3% is unacceptable as they induce physiological and hormonal changes in rodents (Krohn & Hansen 2000). The different commercially available IVC-cages today have different tightness of seals depending on the model. Some are quite open, whereas others are almost totally sealed. Depending on the tightness of the cage, 3% CO_2 can be reached within 20 min, and therefore to keep the welfare of the animals uncompromised, IVC-cages should not be unventilated for more than a few minutes (Krohn & Hansen 2002).

Different studies have shown that IVC-systems can protect rodents against infections and diseases from the outside, if run properly on positive pressure (Lipman et al. 1993, Morrell 1997). In order to keep the animals protected against infections and the staff protected against infections and allergens, the cages must be opened and changing in special environments, e.g. a ventilated bench or a LAF-cabin. The cage functions as a barrier to the surroundings as far as the inside pressure is preserved and the seal is unbroken (Clough et al. 1995). The moment of major risk for contamination of cage and animals is when the seal is broken and the animals are handled. Therefore it is important only to open the cage and handle the animals inside an area with HEPA-filtrated air using aseptic procedures. A standard operation procedure (SOP) must be designed locally for description of procedures used for changing the cages and handling the animals, in order to fit to the equipment used and the facility. The SOP should include information about handling of clean and dirty cages, how to handle the animals, and when and how to clean cages, benches and other equipment during the procedures.

In conclusion, to keep the animals as unaffected as possible, when housed in IVC-systems, a number of factors must be considered:

- The number of air changes must be kept as low as possible, as high numbers of air changes are uncomfortable for the animals.
- The cage should be equipped with bedding material, giving the animals an opportunity to avoid draught
- The cages should not be left unventilated for longer periods, in order to prevent any raise in the level of CO_2 in unventilated cages
- The cages should only be opened in special areas with HEPA-filtrated laminar air flows, and only handled according to the SOP for the procedure

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Figure 1: The first filter top cage developed in 1958 by Dr. Lisbeth Kraft. A metal cylinder with wire mesh walls wrapped with fibreglass insulation and a metal top and bottom.

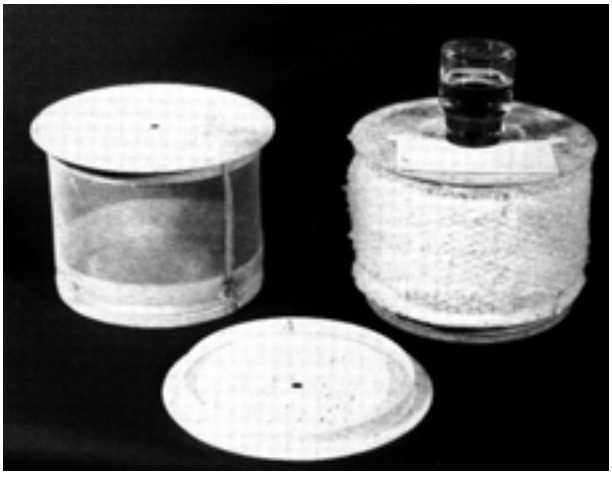

Figure 2: The three different ventilation principles used for IVC systems with filter top cages. A) One ventilator is ventilating air into the cage and diffusing it out through the filter top. Inside the cage is a positive pressure to that of the room. B) One ventilator is ventilating air out of the cage and air diffusing into the cage through the filter top. Inside the cage is a negative pressure to that of the room. C) One ventilator is ventilating air into the cage and one ventilator is ventilating air out of the cage. Depending on which of the ventilators ventilating most air, the pressure inside the cage can be either positive or negative to that of the room, as the air is diffusing either in or out of the cage through the filter top. See text for more details

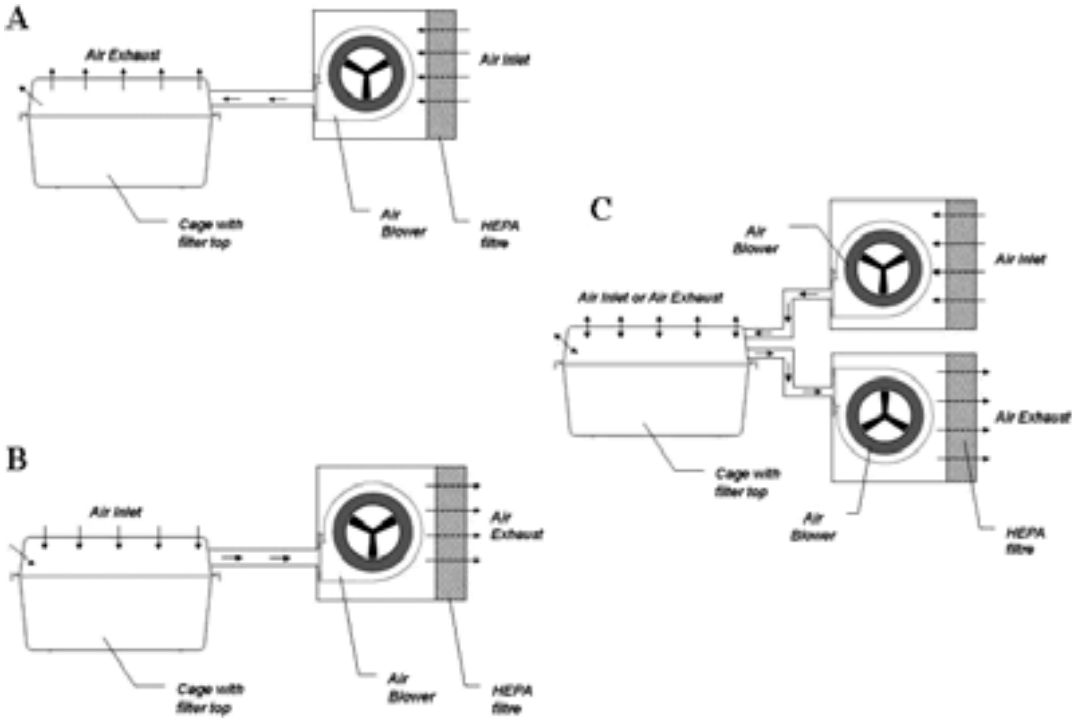

Table 1: The advantages using IVC-systems

Improved protection	By using IVC-systems instead of conventional open cages, the microbial protection of the animals is improved considerably especially when running at positive pressure, and if the system is running in negative mode the protection of the staff against allergens will be improved
Protection at rack level	As the inlet air, when the system is running in the positive mode, is HEPA filtered, it is possible to have a protection at rack level compared to the conventional system where the protection is at room level.
Improved micro climate	Due to the high number of air changes in the cage, the microclimate is improved compared to a conventional cage. There are no progressive CO_2 and NH_3 concentration, and temperature and humidity are kept on the same level as that of the room.
Prolonged periods between cage changing	As the cage is highly ventilated there is no increase in humidity, and therefore bacterial growth and subsequently NH_3 production and the need for cage changing will be reduced.

Table 3: The disadvantages using IVC-systems

Health monitoring problems	In IVC-systems it is more difficult to perform health monitoring, as each cage is protected against the environment. It is not logical to have a sentinel cage in the rack, as these animals are not in contact with the rest of the animals in the rack.
Cage change problems	To maintain a high level of protection cages need to be changed in special benches or in special areas of the room equipped with Laminar Air Flow (LAF). Even if the cage change does not take place in some kind of LAF-unit, it is more time-consuming to make cage changes in IVC-systems compared to open cages as each cage has a lid that must be opened before access to the animals
Requires constant ventilation	The cage requires constant ventilation, as the spontaneous air change is reduced in the modern IVC-cage due to improved filter media and tighter sealing. The animals' respiration causes a rise in CO_2 concentration and will, if not removed quickly, reach harmful levels.

Housing of mice in regulatory toxicity studies in compliance with the new european regulations

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Summary

Improving the housing conditions of laboratory animals is a pertinent issue. Although many previous studies concluded that group-housing and environmental enrichment could contribute to the well-being of animals, it is difficult to select optimal housing conditions based on objective data. Therefore, each laboratory should evaluate the impact of selected housing conditions on the well-being of animals. In the context of regulatory toxicology, it is also essential to determine possible consequences on the conduct and reliability of toxicity studies. In the present study, B6C3F1 mice were housed either singly or in groups in stainless steel or polyethylene cages. Two types of enrichment, a mouse box or a mat made of hemp fibres were compared. The design was intended to mimic a 13-week repeated dose toxicity study. The behaviour, physiology and standard haematology and clinical chemistry parameters were assessed on various occasions. In this study, it was possible to house groups of three male or female B6C3F1 mice for 13 weeks in stainless steel cages but not in polyethylene ones. Although the behaviour of the animals was different between sexes and depended on the type of environmental enrichment, the well-being of group-housed, enriched mice was considered as improved. There were some differences in body weight gain and food consumption, which remained within the normal range. There were slight, if any differences in most haematology and clinical chemistry parameters, except for neutrophil count in males housed in polyethylene cages. Serum corticosterone levels were lower in mice group-housed in polyethylene cages, but adrenal and thymus weights showed no consistent trend. Because mats made of hemp fibres allow for a better observation of the animals, this is the environmental enrichment that is now in routine use for repeated dose toxicity mouse studies in our facilities.

Introduction

There is a growing demand for improving the housing conditions of laboratory animals. This includes a shift from single- to group-housing and the introduction of environmental enrichment as defined by the revision of Annexes of the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS 123). These changes, however, should take into consideration the particular requirements of regulatory toxicity studies (Dean, 1999). Indeed, different housing conditions have been reported to result in changes of the animal's physiology (Tsai et al., 2002), even though inconsistently (Van der Weerd et al., 2002). In addition, it is essential that the selected environmental enrichment is compatible with clinical observations and easy access to the animals.

As male mice are known to be aggressive (Van Loo et al., 2003), group housing may be a challenge for long-term regulatory toxicity studies in mice. The aim of this study was to mimic a 13-week toxicity study using B6C3F1 mice, which is often the preferred strain for long-term studies, such as carcinogenicity studies. The animals were group-housed in two types of enriched environment, whereas the reference group was housed singly in non-enriched cages. Behavioural, clinical and physiological observations were made to define our new standard for housing mice in compliance with the forthcoming European regulations.

Material and method

Animal care

Sixty male and sixty female SPF B6C3F1 mice aged between 5 and 6 weeks at the start of study were purchased from Charles River Laboratories USA. They were kept in groups of 20 in a barrier unit dedicated to rodent toxicity studies in a controlled environment for light (12 h dark/12 h artificial light), humidity and temperature. They received a complete pelleted diet "ad libitum" (A04C-10 from SAFE, Villemoisson /Orge, France) controlled for nutriments, microbiological and chemical contaminants.

Caging

The mice were randomly assigned to 5 experimental groups of 12 males and 12 females and each group was housed in different conditions (Table I).

Group 1 mice were housed singly in stainless-steel cages (surface 214 cm^2, height 12.5 cm). All other mice were housed in groups of 3 males or 3 females. Group 2 and 4 mice were housed in stainless-steel cages (surface 330 cm^2, height: 12.5 cm) and Group 3 and 5 mice in polyethylene cages (surface: 530 cm^2, height: 14 cm).

Two types of enrichment were compared in this study. The first enrichment (group 2 and 3 mice) was a white plastic box (Mouse house, B & K, England) of approximately 10 cm^2 and 10 cm height. The second enrichment (group 4 and 5 mice) was constituted of a mat made of non woven hemp fibres (Beekay Happi-mats, B & K, England). This material was supplied, after irradiation at 25 kG, labelled with a date of production, content information, reference number of physical and chemical check and microbiological check. These mats were replaced every week.

Study design

The design of the study was intended to mimic a 13-week repeated dose toxicity study. Therefore, the animals were handled for a sham oral treatment daily and various parameters were measured on different occasions.

Parameters included clinical observations twice daily, recording of animal behaviour daily for the first week, then at weekly intervals. Special attention was paid to aggressiveness, the presence of wounds and the position of the animals with

respect to the mat or box in the cage. Body weight and food consumption were measured weekly. An ophthalmological examination was performed at the end of the study. Blood samples were taken for measurement of standard haematology and clinical chemistry parameters at the end of the study. These included haemoglobin, mean corpuscular haemoglobin concentration, packed cell volume, red blood cell count, mean corpuscular volume, reticulocyte count, platelet count, total white blood cell count, differential white blood cell count, and serum levels of sodium, potassium, chloride, calcium, glucose, urea, total cholesterol, total bilirubin, total protein, albumin, albumin/globulin ratio (calculated), creatinin, phosphatase alkaline, aspartate aminotransferase, and alanine aminotransferase.

Finally, serum corticosterone levels were assayed at the end of the study, and adrenals and thymus were sampled and weighed at necropsy.

Statistical analysis

Body weight, body weight gains, food consumption, haematology and serum clinical chemistry parameters, and organ weights were analysed separately for males and females. Data from females and males were pooled for the analysis of serum corticosterone levels. Data with homogeneous variances (Levene's test) and normal distribution (Shapiro-Wilk's test) in all groups were analysed using ANOVA followed by Dunnett's test. Data showing non homogeneous variances or a non normal distribution in at least one group were analysed using Kruskal-Wallis test followed by the Wilcoxon's rank sum test. For terminal body weights and organ weights, Kolmogorov's test was used for normality of the data distribution in each group and Bartlett's test for homogeneity of variances across groups, followed by ANOVA and Dunnett's test.

Results

Behaviour

Overall, males were more aggressive than females and this was confirmed by the presence of wounds in some animals. One male housed in a polyethylene cage with the mouse box and 4 males housed in 2 different polyethylene cages with the mat had to be separated and housed singly because of marked wounds seen after 7 and 10 weeks, respectively. In contrast, there were no marked wounds in animals kept in metallic cages. Barbering or focal loss of whiskers was noted in the majority of animals and was seemingly independent of the housing conditions.

Animal position and enrichment

The animal position was enrichment-dependent. Males housed in stainless-steel cages were more often under the mouse box, while females were often lying on the box (picture 1) In contrast, both males and females housed in polyethylene cages were more often under the box (picture 2) Mice housed in stainless-steel cages used the hemp fibres as a mat and were often lying on it (picture 3) In contrast, females housed in polyethylene cages made a nest of the hemp fibres and often hid within the nest, in contrast males made a less complete nest and were often lying on it (pictures 4 and 5).

Once in the study the mice were observed during the night (week 10). They were generally awake and did not seem to pay attention to the enrichment. During the day, however, it was often difficult to observe group-housed animals in the mouse box, whereas they could be easily observed on the mat.

Physiological parameters

Whatever the type of environment, group-housed males had a greater body weight gain than singly housed animals, however their food consumption was lower than singly housed males. For females the singly housed animals had a greater body weight gain than group- housed mice and their food consumption was greater. All of these changes however remained within the normal range of our background control data in this strain of mice.

No unusual findings were noted during the ophthalmological examination at the end of the study.

Haematology and serum clinical chemistry

The only differences between groups were increased neutrophil counts in males housed in polyethylene cages: mean value for group 3: 5.11 k/mm^3, mean value for group 5: 4.09 k/mm^3 when compared to the reference group: mean value 0.60 k/mm^3 ($p<0.001$). There were no differences between groups in any other clinical chemistry parameters.

Serum corticosterone levels

Serum corticosterone levels were statistically lower in mice group-housed in polyethylene cages. Mean corticosterone levels were 111 ng/mL in group 3, 126 ng/mL in group 5 and 326 ng/mL in group 1 mice ($p< 0.001$). Minor increases or decreases in the weight of the adrenals and thymus were seen between groups, but they were not considered as related to the type of environment.

Discussion

The well-being of laboratory animals is difficult to assess, as is the measurement of stressful situations. Although group-housing and environmental enrichment are considered to improve the well-being of animals (Olsson & Dahlborn, 2002), this widely accepted claim is rarely based on objective data (Van de Weerd et al., 1997).

The presence of wounds reflects aggressive behaviour among group-housed animals, especially males. Interestingly, no wounds were noticed in animals kept in metallic cages. Barbering is probably a less specific parameter as a high percentage of mice had a focal loss of whiskers whatever their housing conditions.

Differences in body weight gain and food consumption were noted, which contradicts the previous report that nesting has no influence on the physiology and behaviour of mice (Van de Weerd et al., 1997). However, these were only slight differences within the normal range of our background control data for this strain. Nevertheless, background control data for body weight and food consumption will have to be reviewed for animals kept in the new housing conditions.

Most standard haematology and clinical chemistry parameters measured in group-housed animals showed no statistically significant differences compared with singly housed animals. One exception is the increased neutrophil counts of male mice group-housed in polyethylene cages, the correlated with the presence of wounds this suggests that neutrophil counts is a reliable predictor of the aggressive behaviour of animals.

The mean weight of adrenals and thymus was not different among the various groups of animals, whereas serum corticosterone levels were the lowest in mice housed in polyethylene cages, although they seemed to fight more frequently. As these polyethylene cages were larger than the steel cages, the size does not seem a factor in the aggressive

behaviour.

Although it was difficult to provide objective evidence of the improved well-being of animals using different housing conditions, housing groups of 3 male or 3 female B6C3F1 mice in stainless-steel cages with fibre mats seems to represent a satisfactory condition. Other criteria can be used to make the decision. They include the accessibility to the animals for the technical staff, which is absolutely essential in the context of regulatory toxicity study, and the cost of the enrichment. The hemp-fibre mat is preferred to the plastic house as it allows a better observation of the animals without overt disturbances and the cost is lower.

In conclusion, we were able to house male and female B6C3F1 mice in groups of three for 13 weeks in stainless-steel cages containing either a mouse-house or a mat as an environmental enrichment. This did not prove possible to do in polyethylene cages in the same conditions. Steel cages with group housing of 3 mice and a fibre mat has now been adopted as our standard for long term toxicity studies in mice. Several subsequent mouse studies using this selected environment gave consistent results.

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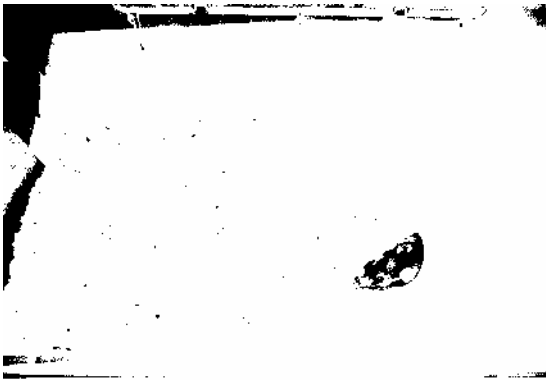
Table 1 Experimental scheme

Group	Housing conditions	Number of animals/cage	Number of animals/group/sex	Number of cages
1	Empty stainless-steel cages	1	12	24
2	Stainless-steel cages + plastic house	3	12	8
3	Polyethylene cages + plastic house	3	12	8
4	Stainless-steel cages + hemp fibres mat	3	12	8
5	Polyethylene cages + hemp fibres mat	3	12	8

Picture 1
Females lying on the box.



Picture 2
Animals in plastic cages lying under the box

Picture 3
Animals in metallic cages with the mat

Picture 4
Females hidden in a nest

Picture 5
Males in a nest.

Effects of housing condition on experimental outcome in a toxicological study

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Abstract

Biotechnical and other experimental routine procedures are believed to have an effect on the outcome of an animal experiment. We started a project to evaluate the effect of these procedures on a range of parameters. Particular interest is given to animal welfare. In this study we focussed on the effects of individual housing of Wistar outbred rats in a toxicology experiment, looking at immune responses, endocrine responses and organ-weights. We found housing condition to affect the bodyweight of the animals. In addition metabolic organ weights of some organs were affected by housing condition, although gender played a more prominent role in these results. No effect of housing condition was found on the antibody production to SRBC immunisation. An important finding is that the effect of chemicals may differ between housing conditions as shown for some organs and T3. This study shows that housing rats socially instead of individual might not interfere with experimental results.

Introduction

Animal experiments are performed to study the response of an animal to a certain experimental procedure. Traditionally researchers attach much value to certain experimental conditions, such as microbiological status and room temperature. The underlying idea is that these conditions might affect the experimental results. However, it may be anticipated that biotechnical and other experimental procedures (accompanying procedures) will have an effect on the outcome of an animal experiment. One can expect that any effect might result in a qualitative or quantitative increase or decrease of the animal's response to the experimental procedure. Therefore, accompanying procedures are considered to be important and increasing interest is given to the effects of accompanying procedures, particularly on animal welfare.

A project was started with the aim of evaluating the effects of several accompanying procedures on experimental results in laboratory animals. These procedures included handling and fixation of the animals, injection techniques, housing conditions and surgical procedures. Several parameters were evaluated such as immunological, behavioral and hormonal responses. This was in order to gain insight in confounding factors and possibilities for refinement in order to optimize and standardize accompanying procedures. The additional idea behind this was that standardization might lead to less variation in experimental outcome and in turn in a reduction in the number of animals that need to be used.

In the study presented the focus was on the effects of social and individual housing, behaviour, immune response, endocrine response and organ weights in a large-scale toxicity study. In statutory required animal experiments the animals are frequently housed individually, according to test regulations. This might be based on the need to monitor individual food and/or water intake and the reluctance to house animals socially. This is based on the idea that any kind of stress induced by social interactions in an experimental animal is undesirable. The influence of social interactions in a research protocol should be considered in the context of the overall response of the animal to accompanying procedures. The responses to stress caused by individual housing may overshadow any possible adverse interactions associated with social interaction. It is also important to realize that the presence of stress caused by individual housing may produce a range of undesirable behavioural or immunological changes that outweigh the effects of social interaction, which may alter the rate of experimental procedures.

Animals, materials and methods

A parental generation of Wistar outbred rats (RIVM: WU(CPB) was dosed, via the Benchmark dose approach (Woutersen *et al.,* 2001; Slob, 2002), with a brominated flame retardant (TBBPA). The brominated flame retardant was added to a commercial pelleted diet (Hope Farms rat chow, Hope Farms, Woerden, The Netherlands). The offspring was kept on the same doses (8 dosages in total) as their mother till the end of the study. The animals received food and tap water *ad libitum.* At the time of weaning the litters were separated by sex. These animals were randomly single-housed in a Macrolon type III cage or social (N=5) in a Macrolon type IV cage. All animals were held under SPF conditions. Some of the males were used in an immunisation study to test the immune response to sheep red blood cells (SRBC). The others were used in neurobehavioral studies. At the end of the treatment period, at the age of 16 weeks, the animals were euthanased by ex-sanguination from the abdominal aorta under CO_2-anaesthesia. The time of killing was approximately the same for each dose-group. Females were sacrificed at the first day of dioestrus. Endocrine and haematological parameters were measured as well as organ weights. Histopathology was performed on certain tissues, but was not reported.

Data analysis

Effects of the brominated flame retardant on the parameters were analyzed by dose-response modeling and estimation of the Critical Effect Dose (CED) (Woutersen *et al.,* 2001; Slob, 2002) by use of the statistical package "Possible Risk Obtained from Animal Studies" (PROAST, version 01). Before further analysis, a Levene's test of homogeneity of variance and a Kolmogorov-Smirnov test of normality was run for all parameters. The majority of the parameters demonstrated normality and equal variances between groups, which made parametric tests preferable. The significance of the differences between groups was calculated by means of Analysis of Variance (ANOVA) (fixed factors:

gender, housing condition and dose-group; covariate: age). If an effect of the brominated flame retardant was revealed the ANOVA was performed within the dose group on housing condition and gender. Otherwise the ANOVA was performed on all dose groups packed together.

A rejection-criterion of 0.05 was set for all statistical tests. If the analyses of variance showed statistically significant effects, the group means were further compared with the unpaired Student's *t* test or with the Bonferoni post-hoc test. All statistics are two-tailed. The statistical package for the social sciences (SPSS, version 9.0) was used for all statistical calculations of significance of differences between the groups.

Results

Bodyweight and organ weight (experiment I) of 40 socially housed males, 15 individually housed males, 40 individually housed females and 15 socially housed females were measured. From these animals blood was collected for clinical chemistry and endocrine parameters.

In a parallel experiment (experiment II) immunological and haematological parameters were determined for 35 socially and 15 individually housed animals that were tested for their immune response to SRBC.

Descriptive results of the above mentioned parameters will be presented, as the work will be published in detail elsewhere.

Experiment I

Body and organ weights

Bodyweight between genders differed statistically significantly at the time of section. Males had higher bodyweights than females. Within females bodyweight also differed significantly between the housing conditions, in which the isolated housed females were heavier than the socially housed ones. To correct for these differences in bodyweight, organ weights have been converted to metabolic organ weights. Analysis of variance has been performed on the corrected organ weights within gender for housing condition.

Based on the corrected organ-weights, within males significant effects of housing condition were found on the thymus and the prostate. Socially housed males had heavier prostates and lighter thymus glands. Within females housing condition had significant effects on the brains and the uterus. Both organs were heavier for the socially housed animals.

For those organs in which the factor doses seemed to have an effect, housing condition had only significant effects in dose group 0, the control group. This might suggest that the brominated flame retardant masks effects of housing conditions.

Endocrine parameters

The endocrine parameter thyroxine (T4) has been analysed separately for gender for the reason that males had significant higher levels. Within gender no effect of housing condition was found.

For triiodothyronine (T3) the males showed no dose-effect and no effect of housing condition. The females had an influence of doses. We found, within dose group 0, the control group, socially housed females have significant higher levels of T3. No housing-effects were found in the other dose groups, which might suggest as well that also for T3 the brominated flame retardant masks the housing effects.

Clinical chemistry

Blood collected at autopsy from the abdominal aorta was used for clinical chemistry. In each sample the following measurements were made: alkaline phosphatase activity, alanine acetyltransferase activity, total protein, creatinine, cholesterol, albumin, glucose and urea. Housing condition had no effect on these parameters. Significant effects of gender were found on albumin, alanine acetyltransferase activity, glucose and on the total of proteins. Except for albumin, males had significant higher levels of the above parameters.

Experiment II

Haematological and Immunological parameters

At the time of weaning 32 males (4 animals per dose group (N=8) were housed socially (N=2) and 15 males (5 animals per dose group (N=3) were housed individually to test the immune response to sheep red blood cells (SRBC). At the age of 11 weeks the animals were immunised with SRBC. At the end of the treatment period, at the age of 16 weeks, the animals were euthanased by ex-sanguination from the abdominal aorta under CO_2-anaesthesia. The time of killing was approximately the same for each dose group. Blood was collected for haematological and immunological parameters.

Haematology

In each blood sample the following determinations were carried out: haemoglobin, red blood cell count, relative distribution width of erythrocytes, relative distribution width of erythrocytes haemoglobin, reticulocytes, total white blood cell count, differential white blood cell count, platelets, mean platelet volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration.

Although there were some dose effects, no housing effects were found on the different types of leucocytes, except for the absolute number of large unstained cells, which were significantly higher in number for the individual housed animals. Housing effects were found on the relative distribution width of the erythrocytes (RDW) and on reticulocytes. The socially housed animals had a significantly higher RDW. Individually housed animals had a higher percentage of reticulocytes.

Immunology

Within bone marrow the differential white blood cells were counted. No effects of housing condition were found on the leucocytes. The NK-activity of spleen cells expressed in % release per spleen culture with regard to the control showed no significant difference between the housing conditions.

Determination of the following Cluster of Differentiation (CD) of lymphocyte subpopulations of the spleen have been performed; CD3, CD4, CD8, CD4/CD8, CD161A, CD45RA. Only significant effects of housing were found on % of CD161A of the total of spleen cells. CD161A was higher in

individually housed males.

Conclusions and discussion

Immune function is believed to be dependent on the well being of the individual. Epidemiological studies have shown that isolated individuals tend to display higher mortality rates than more socially integrated individuals (Baldwin *et al.* 1994). Individual housing of animals that are normally living under social circumstances are believed to suffer from stress. In this study we were particularly interested in effects of housing condition on immune responses, endocrine responses and organ weights of rats.

In our study individual housed animals were heavier than the socially housed ones. This is in accordance with several studies with mice in which reduced bodyweights were found for group housing. Other studies showed no effect of housing condition on bodyweight, while Stefanski *et al.* (2001) mentioned that animals suffering from stress are normally recognized by their reduced body weight. The lower bodyweights of the socially housed animals are believed to be the result of more active individuals due to social interactions and a larger repertoire of natural behaviour as found in a study of Van der Harst (2003). However, instead of bodyweight body composition (body fat:muscle) should be taken into consideration, as it is known that individually housed animals perform stress-induced or boredom-induced eating. In the study of Van der Harst (2003) the time spent on food consumption was equal for both individual and socially housed animals and therefore the amount of food consumed it is not likely to be the reason for the fact that socially housed rats are less obese.

Converting organ weights in metabolic organ weights and taking age as a covariance in the analysis to reduce the variability in these parameters were only partially successful. These findings are in agreement with other fields of toxicological testing. Gur and Waner (1993) performed a toxicological study with organ weights as the parameters of interest. They repeated the study 5 times under the exact similar conditions and using the same stock strain of rats. Great variability between the individuals within a study and between the studies in body- and organ-weight were the results.

In our study metabolic organ weights of some organs were affected by housing condition, although gender played a more prominent role.

Housing condition had no effect on the antibody production to SRBC. This is in accordance with the findings of Stefanski *et al* (2001) and Baldwin *et al* (1995). However in the study of Baldwin *et al* (1995) individually housed animals showed an increase in blood lymphocyte percentage. Further determination has not been done, but our results show that CD161A may play a part in the increase in blood lymphocyte percentage.

It is believed, from the control groups, that the effect of chemicals on parameters may differ between housing conditions as shown for some organs and T3. The brominated flame retardant might outweigh the subtle effects of housing condition for these parameters.

Although there were significant differences within some parameters between the different housing conditions, the differences were very subtle. A major problem in interpreting these significant differences in the presented parameters is the influence of age, bodyweight and gender, which markedly alter many of these variables. The consideration to house rats socially or individually should be based on the purpose of an animal experiment and the sensitivity of differences in parameters that serve this purpose. This study shows that housing rats socially instead of individual might not interfere with experimental results.

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Refining cage change in rats modifications based on telemetric cardiovascular data

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Laboratory animals are regularly exposed to many housing and care procedures, some of which may cause considerable or long lasting disturbance in animals. Cage change is a typical example of such a procedure â€it is usually repeated once or twice a week. Any major disturbance not only compromises animal welfare, but may - if the consequences last long - render animals unsuitable for certain studies for considerable time. The aims of this study were to determine the influence of different cage change modifications on cardiovascular and locomotor parameters of the rat, and to find out which cage change procedure is least disturbing. The study used total of 24 male rats. All rats came with litter information from the breeder, and they were randomly allocated into cages (3 rats per cage, all rats from different families). From each family a single rat was chosen on random basis to be implanted with TA11PA-C40 telemetry transmitters. Four different cage change modifications with crossover design were used, so that each cage received one modification at two week intervals, always at the same time. The control and four different cage change modifications (A - 'move-back to dirty cage'; B - 'all clean'; C - 'clean cage - old enrichment'; D - 'clean cage - old cage cover') were executed on each rat and on each cage. Blood pressure and signal strength were recorded using Dataquest A.R.T. 2.2 Gold system (Data Sciences International, USA). Data were sampled from the transmitters for 10 sec every 5 min for 24 hours before each cage change and continued for 24 hours thereafter. Cage change influence on the cardiovascular system of the rat was evaluated by changes in heart rate (HR), mean arterial (MAP), systolic (SP) and diastolic (DP) pressures and with duration of the changes. Analysis of results revealed that: 1) all cage change modifications caused statistically significant ($p<0.05$) increase in cardiovascular parameters and locomotor activity: 2) the highest increase in HR was caused by the procedure 'clean cage - old enrichment', while the procedure 'all clean' caused the highest increase in MAP, SP and DP; 3) the least effect on cardiovascular parameters was seen with 'move back to dirty cage' procedure; 4) locomotor activity of rats was most increased after the 'move back to dirty cage' procedure, while the 'all clean' procedure was the least disturbing; 5) the longest duration of cariovascular and locomotor response was after an 'all clean' procedure, whereas the 'clean cage - old cage cover' procedure caused shortest lasting responses. In conclusion, transferring the old cage cover seems to be the preferred cage change modification by rats.

Implementation of group-housing in nonhuman primate toxicity studies

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Summary

The regulatory requirements of Animal Welfare support the idea that group-housing in nonhuman primates would result in behavioural benefits in regulatory toxicity studies. The goal of this study was to investigate the practical issues of group-housing in toxicology by evaluating environmental, social, technical and computerization issues.

Structural environment was enriched by using connecting doors, shelves, toys, progressive light intensity systems and radio sound generators. This increased opportunities for the expression of species-typical behaviour and activity and enhanced the animals' well-being.

The implementation of a selective grouping of socially compatible primates in agreement with the protocol design reduced behavioural abnormalities (i.e. stereotypes), increased opportunities to exercise and expanded cognitive stimulation between grouped animals (i.e. grooming and huddling).

Technical adaptations included additional body and cage identification systems to ensure individual identification among grouped animals. Randomisation was reinforced based on both compatibility of paired animals, bodyweights and clinical parameters. Animal confinement was limited in time based on specific study requirements (e.g. post-treatment digestive clinical signs).

Computerized adaptations consisted of testing switches between paired animals within a group with corresponding raw data transfer and in validating two types of clinical sign recordings either for singly or paired housing with corresponding adapted reporting.

The main concerns were related to the evaluation of gastro-intestinal clinical signs and individual food consumption estimation. As these 2 parameters were rarely considered critical for assessing toxicity, they did not jeopardize study results. The main benefits were stable social paired animals that were calmly and spent more time interacting.

In conclusion, all of these tested group-housing strategies resulted in behavioural benefits for cynomolgus monkeys without compromising the regulatory requirements of the studies. Thus, these measures have been implemented in routine nonhuman primate toxicity studies.

In recent years many efforts have been done to promote housing, care and behaviour of laboratory animals in toxicological research (Dean 1999, Bayne 2003), especially in nonhuman primates for which there is growing insight to provide opportunities for species-specific behaviours (e.g., feeding and environmental enrichments, self- and social behaviours) (Line 1987, Novak & Suomi 1988, O'Neill 1988, Fajzi *et al.* 1989, Bryant *et al.* 1988, Watson *et al.* 1989, Line *et al.* 1990, Bernstein 1991, Watson 1992, Brinkman 1996, Röder & Timmermans 2002). Concomitant to these efforts, international regulations and recommendations emphasize the need to maximize primate welfare during their scientific use. In 1999, the United States Department of Agriculture (USDA, 1991) specifies that a physical environment adequate to promote psychological well-being of primates should be provided. In 2003, the European council (European convention of the protection of vertebrate animals used for experimental and other scientific purposes, 2003) describes the current practices and future goals for incorporating refinements into housing environment for nonhuman primates used in toxicology research. It recommends that nonhuman primates should be housed with one or more compatible congeners. Attention should be paid to the age and sex composition of the groups and animals should be carefully monitored after grouping for minimising possible aggressive interactions. The only exceptions to group-housing should be either for veterinary reasons (e.g., poor clinical conditions or fighting injuries) or where an experimental study plan demands it to ensure good science (e.g., follow up of treatment-related clinical signs in individual, toxicokinetic profiles). Overall, single housing should only be allowed for as short time as possible, under close supervision such that re-introduction would not disturb the social organisation of the group. However in case of conflicts, possible solutions include confinement of this animal to an individual cage attached to, or within, the main area or separation of all individuals briefly followed by re-introduction of the whole group simultaneously.

Despite on these European recommendations, there is some reluctance from toxicologists to implement social housing of nonhuman primates in regulatory studies because of the scarcity of data reported in literature and the lack of historical data on studies performed in group-housing. Thus, it is important to balance welfare and enrichment enhancements with the high-quality science of carefully controlled Good Laboratory Practice (GLP)-compliant toxicological studies, by minimising the impacts of confounding variables such that studies could be not jeopardized. Considering requirements in terms of number of animals per group and per sex that usually ranges from 2 to 5 individuals in a 2 to 4-week standard toxicity study, corresponding to pivotal toxicity studies for the first in man, there are concerns to form adequate and compatible groups of animals pending on the end of dosing and recovery period designs. Moreover, several parameters should be evaluated to select appropriately animals in groups before the treatment initiation (e.g. bodyweights, clinical pathology data, cardiology data, ophthalmology data) such that there are concerns about the constitution of homogeneous grouped animals and the possibility to switch animals intra- or inter- groups before start of dosing without

any risk of incompatibility between already formed groups. This necessitates having enough supernumerary animals to be able to re-order grouped animals based on both clinical data obtained and compatibility between congeners.

Risks of incompatibility within a group during the study should also be considered. For example, consecutive injury from fighting, social distress or undernourishment of subordinate animals among a group (Crockett 1990, Gust 1993) may conduct to a temporarily or permanently removal of an animal from a group. This isolation may induce differences in physiological and/or behavioural responses that could impact on data evaluation because all animals of a dose level group will not be in the same environmental conditions. Based on previous study results, key parameters related to the pharmacological or toxicological effects of a drug may require to be followed up individually (e.g. post-dosing gastro-intestinal clinical or central nervous system signs) during an defined part-time isolation. Issues may raise from resulting data that should be recorded differently than those taken into group-housing. The last concern of toxicologists refers to changes such as ingestion of faeces and /or substrate enrichment that may confound the study results by risk of ingestion of compound or its metabolite(s), or ingestion of material of unknown composition (Dean 1999).

Overall, number of pharmaceutical laboratories already have ongoing environmental enrichment programs dealing with husbandry cage structures, climatic or light adaptations, substrate and/or foraging adaptations in compliance with Good laboratory Practice of toxicology research. These adaptations were guided from the integration of increasing insights into the side-effects of poor housing and care conditions on behaviour and physiology of nonhuman primates on the one hand, and by insights into their natural life in wild and reported benefits of experiences performed in enriched housing conditions on the other hand. However, there is few information on housing of Cynomolgus macaques in groups for toxicological studies (Dean 1999, Bayne 2003). Thus, the aim of this research was to investigate the practical improvement of group-housing in regulatory nonhuman primate toxicity studies. Based on last European regulations and recommendations (European convention of the protection of vertebrate animals used for experimental and other scientific purposes 2003) and in taken into account concerns of toxicologists about social housing implementation in toxicity studies, group-housing of cynomolgus monkeys was evaluated using simulated computerised studies and tested into a 2-week exploratory toxicity study. Data of these studies not reported here, served to support four key strategies that will be developed hereafter and that include enrichment, social compatibility, technical issues and computerized issues.

Environmental enrichment

Providing a large and stimulating place to live for captive nonhuman primates was the first developed strategy to enriching their environment. For this purpose, several equipments were installed. In order to allow social contact for much of the time , individual cages were adapted to be interconnected between them by opening transversal doors such that it was possible to house grouped animals from two to three on the same rack. The size of these connecting doors was adapted to allow low-ranked animals to have the possibility to get out sight of high-ranked group members. In addition, elevated and large platforms were added as this is frequently the most used enrichment device in a variety of non-nutritive/non-social enrichment (Bayne *et al.* 1991, Reinhardt 1995). Such structures may provide a sense of increased security from being off the cage floor and allow primates to choose between different elevations in the connected cages. Different (e.g., plastic blocks with holes drilled in the middle, stainless steel trays and bowls, hard rubber dog-toy, nylon balls) toys of well known chemical composition were also provided, all of them being selectively chosen to be safe, sanitizable and not interfere with toxicological implication. In order to mimic the sun's cycle, progressive light intensity systems were used. Radio sound generators were installed in all rooms of primates as it was observed that animals appeared calmly when listening music.

According to the observations performed during our study, advantages of these environmental enrichments can be summarised as follows. Primates housed in group had a higher exploring and activity levels, the connected cages offering to them larger space to move. The connecting doors gave them the opportunity to break eye contact, allowing the possibility to limit physical contacts, when hierarchy behaviours may occur. Primates spent more time in recreative and foraging activities (e.g., searching patterns, food processing, and consumption). They preferred toys that can be manipulated and carried. They had expanded cognitive stimulations, the most regularly observed interactions being social grooming and huddling. They did not show any sign of depression whenever in groups where there is low-ranked group members. They exhibited lower levels of abnormal behaviours, ranking from active whole-body, self-directed stereotypies to self-injurious behaviour, than do usually singly caged monkeys, ranking from active whole-body, self-directed stereotypies to self-injurious behaviours (Bryant *et al.* 1988, Watson 1998, Röder & Timmermans 2002, Lutz *et al.* 2003).

Only two environmental enrichments' disadvantages were noted. Firstly, primates had a low interest in toys. Whatever the type of toys provided, they were used infrequently and appeared to provide less of an environmental improvement than social companions. Secondly, higher tension-related behaviours were observed in mature adult males than in younger males or than in females, confirming the importance of age and sex for group-housing (Crockett *et al.* 1994).

Based on these provided environmental enrichments, several strategies were consolidated to be implemented routinely in further toxicity studies. The connecting doors system was installed for all cages because it offers two advantages. As this system can be modulated, it could be possible to house the animals by pair or by trio, that is interesting because of possible multiple study designs and part-time isolation requirements. More than offering to animals a larger recreative area, this system also gives to primates the opportunities to be out of sight of another one. With the available platforms, it also provides multiple escape routes to monkeys to avoid attacks and also prevents dominant individuals from restricting access of subordinates to other parts of connected cages. Due to the low interest of monkeys to the proposed toys, it has been suggested to test if a schedule of toy-removal and re-introduction would prolong their interest to use them. Further essays are also performed to introduce more attractive toys. For example, it was shown that shapes and substance of toys may play a significant role in their effectiveness in cynomolgus monkeys. The ring and the translucent flexible plastic were found to be the most effective to elicit manipulation (Weld *et al.* 1991). Other approaches consisted in modifying foraging devices as food puzzles (Reinhardt, 1993 a, 1993b) such that monkeys spend

more time acquiring it. The last implemented environmental enrichment consisted in providing the same radio sound in all primates rooms, internal essays being conducted to evaluate what type of music being the most appropriate for cynomolgus well-being.

Social compatibility enhancement

Facilitating socialisation of nonhuman primates was the second developed strategy to enriching their environment in captivity. To this issue, a constant clinical and social monitoring of grouped animals was performed from their arrival to the initiation of studies. In toxicity studies, most of the group sizes are 2 or 3 large monkeys. Thus, animals were housed per trio as it is easier to dissociate a trio than to add a new companion to a pair in case of groups in study of 3 animals for end of dosing and 2 remaining animals for end of reversibility period.

In toxicity studies, before start of dosing, a period, named pretest, is required to select animals for the study based on evaluated parameters including clinical pathology, electrocardiograms and ophthalmology. As these parameter results may compromise the already formed groups of monkeys, additional groups of supernumerary animals were added. This pretest period was extended to 2 weeks, the first week being dedicated to the evaluation of parameters and the second week to the observation of grouped animals, some of them possibly having to be switched inter- or intra- groups because of elimination of animals presenting non acceptable spontaneous anomalies or being not in healthy conditions (e.g., abnormal cardiac profiles, disturbed blood formulation).

According to the evaluation of our study, the main advantage of this progressive facilitated socialisation of nonhuman primates was to obtain stable grouped animals at start of studies due to their continuous clinical and social monitoring from their arrival to husbandry. Grouping animals per trio offered the advantage to manage more easily the study groups, pending the number of animals required per dose and the study design with or without a reversibility period. Overall, the manipulations were facilitated because monkeys were calmly and easier to handling when compared to singly housed animals.

Social compatibility enhancements had only three minor disadvantages. Firstly, more animals are temporarily used in the pretest period since supernumerary animals should be considered as a trio of animals and not as supernumerary individuals. Secondly, the study directors will preferably select them as groups for the study as they are socially compatible than to have to re-order already formed groups with the risk of disturbing their compatibility. Thus, the group-housing could be a source of limitation of the study animal selection. Finally, these group-housing studies have a 1 week longer duration to ensure the stability of grouped animals before start of dosing. Since such design always will be anticipated when scheduling the studies, it will not really impact on the compound development delays.

Based on the study results of these social compatibility enhancements, several strategies were consolidated to be implemented routinely in further toxicity studies. In order to obtain more stable and homogeneous group-housed primates, it was decided to house primates in groups from their arrival to husbandry, based on their mean bodyweights (usually ranked in the 2-4 kg range at arrival). As the age and sex are known to be key factors influencing the socialisation of cynomolgus monkeys due to their territorial dominance hierarchy habits in wild (Röder 2002, Crockett 1994), it was decided for a same arrival to group animals of the same sex based on their range of age, juvenile/young adults being more compatible than mature adults (males especially). This strategy is in agreement with previous studies that showed that providing social companions results in behavioural profiles indicative of improved well-being in juvenile macaques, in males especially (Line 1987, Novak *et al.* 1988, Dean 1999). On the other hand, although animal wounding is a possible consequence of inappropriately grouped animals, self-injurious behaviours may also occur as the result of signs of depression consecutive to indecisive fighting between dominants and subordinates (Bryant 1988, Crockett *et al.* 1994, Watson 1998, Lutz *et al.* 2003). To prevent such situations during the acclimatisation period, the animal care was in charge of performing daily clinical and social monitoring of the animals to ensure no degradation of their compatibility. As the final study design is often not fixed at arrival of primates, it was also decided to group them arbitrarily per trio, this solution allowing more possibilities to further re-order the animals per pair or per trio. Finally, the choice to extend the period of pretest to 2 weeks was considered as an additional security to ensure compatibility of groups in case of animals' switching due to incompatible clinical data for study requirements.

Technical issues

Technical issues were the third developed strategy to implement group-housing of nonhuman primates without compromising regulatory study requirements. Group housing pushed technicians to find original identification systems that could facilitate the individual identification of the animals when they are housed in groups. As animals should be housed individually for some parameters follow up, it was accepted to keep them in the cage where they were at this moment, independently of the order they have in the study group. To identify them visually rapidly, it was decided to shave selective small parts of their harms, in using up to 3 possibilities, pending on the number of animals in the same cage. In addition, the corresponding animal study number was temporarily written on each individual cage.

As explained previously, animals are selected for the study based on their bodyweights and clinical parameters. The computer randomisation allowing this selection, was reinforced to take into account the compatibility of grouped animals. In order to facilitate social compatibility, such selection could affect a suitable inter-group homogeneity.

Finally, confinement of the animals in single cage was reconsidered and limited as far as possible to specific study requirements in order to minimise risk of conflicts when they return to their original groupings (Gust 1993). Several parameters require such isolation. The most important, because of toxicological concerns, are the post-dosing digestive signs (e.g., emesis, diarrhea) that should be related to the absorption of the compound and may confound data interpretation if not appropriately followed individually. Toxicokinetic profiles should preferably be followed individually as temporarily isolation of animals would considerably reduce manipulations if we consider that up to 6 time-point toxicokinetic samplings could be performed on the same animal within 6 hours post treatment. Other concerned parameters include ophthalmology and electrocardiograms, as the animals should be immobilised temporarily for these examinations.

According to our study observations, advantages of these technical enhancements firstly consist in an easier

identification of individuals among a group such that risk of identification errors is considerably reduced. Secondly, groups of study are composed of more homogeneous animals as the compatibility component has been integrated in the randomisation process. Thirdly, there is no clinical sign information missing as they are planned to be followed individually after treatment with respect of the known clinical pharmacological or toxicological effects of the compound.

Two disadvantages should be considered. Firstly, the daily part-time isolation of animals after treatment induces more manipulations that require high level of technician skills to manipulate primates without risk of biting. Secondly, in addition to the visual body shaving identification, the individual body-tattoo of animals should be systematically controlled before the first manipulation after isolation in compliance with the GLP identification requirements.

All of presenting solutions to solve these technical issues for group-housing received agreements of study directors to be implemented in toxicity studies. They were considered to be efficient to ensure individual identification of animals among a group. Reinforced randomisation based on group-housing compatibility was considered to be a plus for the homogeneity of animals in study groups. Finally, the confinement of the animals was reviewed as minima to cover key clinical data recording related to the compound activity with not disrupting a lot the established socially groups.

Computerised issues

Computerised issues were the fourth developed strategy to implement group-housing of nonhuman primates without compromising regulatory study requirements. Most of the data collection system used in toxicity studies require traceability when animals are switched to a dose group and/or a cage. Switching animals within a dose group in case of incompatibility of pairs or trio should be validated at two levels: firstly, at raw data level, the computer system should automatically allow the good raw data correspondence; secondly, at cage order level with corresponding animal study number, the computerised system should allow to re-house animals within a study group. As clinical signs are recorded at animal level, during the period where the monkeys are grouped, it was decided to attribute artificially the clinical observations of the group to the first animal of each pair or trio. Raw data edition for the report should then be adapted in considering clinical data per pair or trio, pending on how the animals are group-housed. Specific glossaries of clinical signs also were created to be selected whenever animals are followed individually or grouped. For example, food estimation was followed at a group level as it was not considered to be a relevant data when followed individually because of the permanent food spillage operated by the primates.

Based on our study observation, the first advantage of these computerised solutions was that inter-and intra-group animal switches were validated such that there is no possible missed information after computer switches. The second advantage is that technician could adapt its type of data recording whenever primates are followed individually or in group with corresponding secured data edition.

One of the disadvantages to these computerised solutions is that the parameters' follow up is linked to the type of recording at animal or cage level and thus technician should carefully select the corresponding clinical signs glossary when he has to record clinical observations individually or for grouped animals. The other weak point is related to the type of recording allowed by the computerised system. Since some parameters could only be recorded at animal levels such as clinical signs even if the clinical observation may concern several animals, it would be preferable to follow up the spatial housing of the animals during all the study in order to be able to make correspondence between the first animal per cage in group-housing and the corresponding grouped clinical observations. This is of great importance when animals should be temporarily or permanently isolated or when a non scheduled mortality is observed during the study.

These presented computerised solutions were accepted by the study directors as they did not compromise the data interpretation. The use of switch to re-order the animals and /or re-housed functions was routinely implemented in toxicity studies without compromising the corresponding data transfers. Two types of clinical signs glossaries were successfully implemented that were adapted to the expected clinical observations pending on the type of recording in individual or in group-housing. Finally, the manual follow up of the spatial housing of the animals was considered to be an additional security to verify correspondence of raw data after cage switches or after temporarily or permanent individual housing.

Conclusions and perspectives

Based on the mentioned study experience, group-housing of nonhuman primates in toxicity studies was shown to enhance well-being of primates in normalising their behaviour. The selection of the animals for groups is greatly facilitated as long as animals are selected from their arrival to husbandry, based on their compatibility in terms of age, sex and weight. Nonhuman primates in group-housing are calmly and easier to handle for manipulations as the result of more expanded social behaviours such as grooming and huddling and a decrease in abnormal patterns such as stereotypes. In addition, animals are in healthier conditions due to less observed self-injurious behaviours.

Based on the analysis of present strategies, all proposed solutions received agreements of study directors as they were compliant with GLP and did not compromise regulatory toxicity study requirements. The weak points are related to the lost of some individual clinical signs, such as gastro-intestinal signs, and increase of the workload for handling animals. In thinking of other possible constraints of nonhuman primates group-housing in toxicity studies, anticipated solutions could be suggested. Firstly, when a temporarily isolated primate should be re-introduced to the group, progressive visual and grooming contacts bars could be a useful intermediate step to ensure its re-introduction with the resting group (Crockett *et al.* 1997). This strategy would help to avoid aggressive hierarchy events that could lead to physical injuries between congeners (Gust *et al.* 1993). Secondly, when a nonhuman primate should be isolated permanently, critical analysis of data at cage and animal levels should be performed within the group to avoid bias in data interpretation. Thirdly, when key clinical signs should be individually followed accurately, an appropriate part-time isolations schedule should be established before start of study, based the known pharmacological and/or toxicological effects of the compound; and then could be rescheduled pending on clinical signs appearing, progressing and/or regressing with time.

In conclusion, group-housing strategies developed in this research resulted in behavioural benefits for cynomolgus monkeys without compromising requirements of studies.

Thus, part-time housing will routinely be implemented in regulatory nonhuman primate toxicity studies in taking care to schedule appropriate limited isolation periods to ensure key parameters follow up.

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Environmental enrichment for aquatic vertebrates

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The term 'aquatic vertebrate' includes a wide diversity of species which occupy a huge range of natural habitats. However few of these are commonly used in the laboratory - principally fish and amphibia. A small number of these two classes is used in considerable numbers, and these principally for investigations into developmental biology. This presentation will restrict itself to the amphibia Rana temporaia, Xenopus laevis and the zebra-fish, Danio rero.

Of all the common laboratory species, we probably know least about the environmental needs of these animals. The expert Working Parties of the fourth multilateral consultation which are preparing proposals for modifications to Appendix A of the European Convention have recognised a general lack of knowledge in this area. We believe the central nervous system of these animals to be relatively undeveloped; this may have the consequence of curtailing the animals' awareness of deficiencies in their environment, but might also be argued that it limits their ability to cope with such deficits.

It is a relatively simple matter to introduce objects into tanks in which aquatic vertebrates are housed and by simple observation to determine what use made of them. This provides a simple but practical means of determining whether such inclusions are recognised by the occupants and whether they are seen as attractive or aversive. In general it is found that most species avoid bright light and seek shelter and generally darkness. This paper will describe the impact on animal behaviour of introducing a variety of inclusions into the environment of such animals and will examine the practicality of these. Where objects provide concealment for animals it may be more difficult to clean tanks or to examine or capture the animals and these disadvantages could have an overall negative impact on the animals' welfare. Simple strategies for avoiding such problems will be suggested.

Ask the animal! The use of commercially available environmental enrichment by laboratory mice

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In the field of biomedical research, the demand for standardisation of environmental enrichment for laboratory animals is growing. For laboratory mice, a wide variety of environmental enrichment items are commercially available. Most of these comply with the demand for standardisation, hygiene and ergonomics. Whether these items also comply with their actual purpose: to enhance the well-being of the mice, is often not assessed scientifically. In this study, we tested the preference of 15 groups of 3-4 mice (N=49) from 3 different strains for two commercially available nest boxes differing in shape and material: the Shepherd Shack/DesRes (SS/DR) and the Tecniplast Mouse House (TMH), in a simple preference test. To measure strength of preference, both nest boxes were also tested against a highly preferred nesting material. Preference for the most preferred nest box was investigated further in an automated preference test in which 24 mice were tested individually. Results indicate that mice strongly prefer the SS/DR, but not the TMH for nesting and sleeping. When tested against nesting material, mice almost always dragged the nesting material inside the SS/DR, even though they had to work to achieve this. The TMH, on the other hand, was never combined with nesting material. More elaborate testing of the SS/DR in the automated preference test system confirmed that mice spent significantly more time in a cage in which a SS/DR was provided. Differences between both nest boxes are discussed with regard to their attractiveness to mice. The SS/DR is more manipulative and as such may provide an increased environmental control for the mice, which can be regarded as an important behavioural need. It is also argued that enrichment should primarily be developed in concordance with the animals' needs prior to marketing enrichment tools.

Harmonization of rat enrichment

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Introduction

Enrichment strategies may take the direction of inserting any kind of item in the surroundings that is expected to increase the welfare and reduce the boredom. Different laboratories choose different approaches for their enrichment routines. Working towards a common practice for basic husbandry guidelines for enrichment is needed, in order to avoid the introduction of unnecessary variation in results between laboratories. One approach to choose how to enrich is to use the knowledge of the natural behaviour of the rat and the rats' preferences as a guide.

The shelter

Macrolon IV cages (Scanbur) with a floor space of 1848 cm2 are commonly used for socially housed rats. It is a challenge to equip this size cages with enrichment devices considered necessary to enhance the welfare of laboratory rats, without being able to change cage dimensions. Studies at our laboratory indicate that shelters that fill up a relatively large space of the cage are one of the best used items of enrichment you can give, as was judged by the time spent being in the shelter, in the numbers of visits to the shelter, and reduced aggression levels (Jegstrup and Ritskes-Hoitinga, 2002). Manser et al. (1998) also demonstrated the preference for shelters, by showing that rats are willing to invest quite some working effort in obtaining a shelter.

The shelter is not only used as a common nest place (Jegstrup 2002, Jegstrup et al. in print), it also offers the animals a refuge when they become frightened by external influences. In addition, the shelter will divide the cage in several "isolated" compartments. According to our findings, the shelter should preferably have two openings (Pic.1), so a dominating animal never can trap a subdominant animal. It should also have a considerable size (length 22 x width 18 x height 15 cm, the thickness of the wood: 1 cm; beach wood was used, as this is known not to interfere with experimental results, in contrast to some soft type woods), because nests will become elaborate and the shelters should be able to contain both the nest material and the rats that share these (Jegstrup et al. in print). The above-mentioned shelter has been tested successfully in rats of several inbred strains (Jegstrup et al. in print) as well as in SD rats in groups of two that could weigh up to 600 grams (Jegstrup 2002). The measures of the shelter were based on the average size of nests made by wild rats. Adaptation of the size has been done in accordance to the rat strain used and the intended number of rats housed in each cage. Wild rats are on average of a smaller size and have a shorter life span, which implies a lower terminal body weight, as compared to laboratory rats. It was considered important that the shelter had to be made of (hard) wood, as this will eliminate the need for adding any other wooden gnawing material to the cage, and because wood works as a relatively good sound insulator, i.e. much better than plastic and metal materials. By using hard wood, the shelter can be used for long periods of time, despite gnawing. Sound insulation is important, as there can be a lot of noise in the animal unit. By using these types of shelters, the breeding results in rats improves; the GK/Mol strain increased drastically in relation to the average published breeding success of this strain (unpublished observations).

As the animals in our laboratories do not choose their own companions and cannot escape from a nasty cage mate, the shelter provides the possibility to avoid visual contact. As aggression is triggered by visual cues, the shelter can be used as a means of adding space for avoidance of visual contact, without adding extra physical space. By placing the shelter in the centre of the cage instead of other locations, the optimal number of areas for "non-visual compartments" is achieved (Fig. 1.).

The possible aversive effect of applying a shelter to the cage could be the risk for getting more aggressive animals that are more difficult to handle as a result of the occurrence of territorial behaviour and less visual contact with humans. However, as long as it is made sure that animals are handled and trained on a regular basis, the empirical finding is that this does not occur. It has been examined for laboratory mice as well, and it has been shown not to occur for this rodent either (Moons et al, 2004).

Nest material

Nest making is an innate behaviour and is not only performed by female, but also by male rats (Jegstrup, 2002, 2005 in print). Upon applying twigs, leafs, straw, wood wool and/or paper, rats will use these materials for building nests. In the wild, grass or even stalks of weed have been reported to be used for this purpose. It was found that male rats of three inbred rat strains will always build nests, when giving shelters and the proper nest building materials, which illustrates how important this behaviour is for this species (Jegstrup et al in print, 2005). The shelter is an essential enrichment device and combined with nesting material, it will give the frame for the construction of a "real" nest. Rats were observed to have nest building behaviour on a continuous basis, and even though nests were cleaned out every third week, rats kept on rebuilding nests for the total duration of the study which was as long as 6 months (Jegstrup, 2002).

There is already a wide variety of nesting materials available commercially and no decision has been made on what the best option is. From the point of view of manipulative ability, stalks in the form of straw or hay (the latter will probably prove to be the better of the two) has a better usage than paper, and was also the preferred material for the rats during the nest building study (Jegstrup, 2002). In nature, rats line their nest with a soft material like fresh leaves or grass. It is thought that the best standardisation of nesting material would probably be a combination between good manipulative material, together with a soft material, such as paper or seaweed. This is being studied presently.

Bedding

Rats paws have been adapted to relative soft and smooth soil, and are therefore easily damaged on coarse and splintery bedding types like wood chips. Bedding types need therefore to be chosen while taking these factors into consideration. Seaweed bedding used in the agricultural setting has been reported to be effective ammonia absorbent. Besides that, it is a soft and light material, which may be suitable for the laboratory setting as well. However, the material needs to be the analysed for nutrient and contaminant contents to ensure that no negative interference with laboratory studies occurs.

Standardisation and enrichment

It is important to realize that rats' priorities are not absolute, i.e. there may be general patterns for the species rat, but there can also be differences between individuals and strains. Rats from the Brown Norway strain would normally spend some time on the roof of their shelter, however they were reluctant to do so when the shelter was covered with aspen bedding, which was different from the other two inbred rat strains tested (LEWIS and BDIX strain) (Jegstrup, in print). BN rats would only draw in straw provided on the cage lid, in case the amount of straw inside the cage was not sufficient to satisfy their nest building behaviour.

Our understanding of the behavioural and enrichment needs of laboratory rats must go beyond the generalization of the species. Different strains behave very differently towards the same enrichment, and before we implement a standardized enrichment routine as common husbandry procedure in our laboratories we have to realize that species specific behaviour might have changed with the change of genetics. The BDIX rats hardly used the aspen gnawing stick provided inside the cages, even when no other enrichment items were present, whereas Lewis rats always used these sticks independent of other enrichment devices given. By using a wooden shelter, the need for a gnawing stick is eliminated, as those in need for some hard material to gnaw on will have it available at all times, whereas those that do not appear to need these gnawing sticks (BDIX), are not provided with an item of no use.

Conclusion

As a starting point for rat enrichment, the above-described shelter is a valuable enrichment item. Further study is needed on which nesting materials should be applied in addition to these shelters. The current lack of scientific knowledge on the most optimal type of nesting material and cage size, and how different strains and individual rats will respond to this is of concern: current legislation describe enrichment as a necessary action, but does not define in detail how to achieve this. This leads to the risk that great variation in enrichment strategies is chosen at different laboratories, leading to uncontrolled variation in results. It may even lead to increasingly stressed animals, as aversive enrichment items are chosen. Further study to obtain fundamental scientific proof on how to apply harmonized enrichment in the most optimal way is highly needed.

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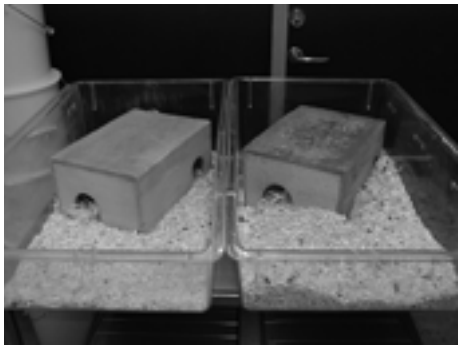

Pic.1. From Jegstrup et al. 2005(in print). Cage and House

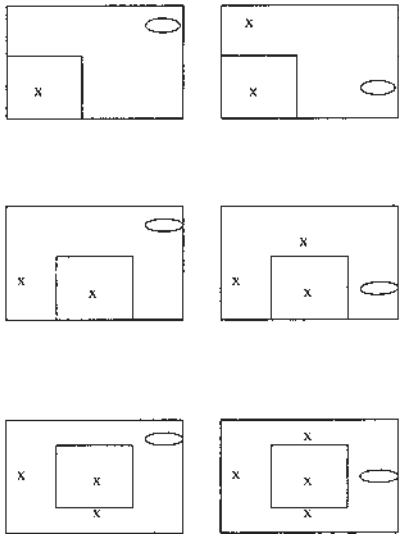

Fig. 1. House, cage mate, non visual compartments for the rat (x)

Running wheels for mice: enrichment or frustration

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The beneficial effects of enriched environments rely on the optimal setting that would enhance the wellbeing of the animals and not simply maximizing housing features which may contrive variant consequences. In spite of its frequent use in research with laboratory rodents, a connection between running wheel, housing conditions and animal well-being was not shown and the value of the running wheel as an enrichment device is not clear. The aim of this study was to find out whether providing the mice with an enriched environment, meeting the animals' needs, would decrease the wheel running activity. Two groups of BALB/c mice (n = 48) age 8 weeks were housed in an enriched condition (EC, Shepherd Shack, two tissues and two aspen wood gnawing sticks) or in a minimal condition (MC, only bedding). The mice were further exposed to three different housing conditions. One group had access to running wheel cages (EC-W; MC-W) on alternating days for 3 weeks, the second group of mice from EC and MC was exposed to individual cage condition (EC-I; MC-I) on alternating days during the same period to control for any impacts of isolation and the third group of EC and MC mice was kept in group housing condition (EC-G; MC-G) throughout the study period. After three weeks of alternate wheel running days, total running activity showed a trend of higher running level in the MC than the EC groups. Running wheel preference was also examined for 4 days by providing tissues as nesting material, a known preferred feature, in the running wheel cages. MC group continued to show a tendency to run more than EC group, but no effect of nesting material in the running wheel cage was found. Endogenous rewarding aspect of wheel running behaviour was examined by acute injections with Naltrexone, an opiate receptor antagonist. Wheel running levels were similar in both groups. Thus physical activity of wheel running did not reveal an endogenous reward system Significant differences in behaviour could be found between animals exposed to intermittent single housing and those exposed to running wheel or group conditions when spontaneous activities were assessed. Both EC-I and MC-I mice exposed to single housing on alternate days moved longer distances, spent more time in a defined center zone of the open field, and had higher velocity. ECI and MC-I mice also showed significantly lower body weight than the other groups. Thus, in contrast to exposure to single housing with running wheel, intermittent exposure to single housing condition without stimulating activity had consequences on the animal's spontaneous activities and body weight.

Ethoexperimental approaches to domestication and animal welfare in the house mouse

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Laboratory animal welfare research has mainly focused on home cage behaviour, preference tests related to different enrichment items, and physiological markers of stress (1). The aim is often to increase opportunities for a natural behavioural repertoire. The aim of the present project has been to introduce a novel approach to welfare assessment through the use of a battery of behavioural tests of exploration, risk assessment and anxiety. The ability to gather information and assess risks in novel environments is crucial for survival and fitness in the wild. There is reason to assume that exploration, risk assessment and anxiety-like behavioural traits are related to the individual's ability to adapt to the environment including laboratory housing and experimental conditions. This ability may not have been selected for in the breeding of laboratory mice. As a basis for this research line we have investigated differences in the behaviour of wild house mice versus two laboratory strains. Wild house mice (*Mus musculus musculus*) was captured and then bred in the laboratory. The behaviour of both male and female adult offspring was characterised and compared to the behaviour of BALB/c and C57BL/6 mice. In total 40 males and 44 females were included. Three behavioural tests were used: The Concentric Square Field, a modified Open Field and a conventional Elevated Plus Maze. In addition to spatial measures also behavioural measures of exploration and risk assessment was registered. We did not find any behaviours that were unique for wild mice but there were data indicating that wild mice, especially males, qualitatively differ from both laboratory strains in being more systematic and rational in their information gathering strategy towards aversive areas. They are cautious before entering a potentially dangerous zone but explore it thoroughly if assessed as non-risky. Furthermore, they do not avoid any zones entirely and employ also the arena edges in their exploration of the novel arena. The wild mice also had a higher avoidance of open areas than laboratory mice. Wild mice differed from both laboratory strains in these parameters but laboratory strain differences were also found. BALB/c mice showed a higher avoidance and risk assessment than C57BL/6 mice, which were more explorative and risk taking than the BALB/c mice. The differences in information gathering quality found between wild and laboratory mice may indicate a higher coping ability in wild mice. The general reduction in defensive reactions seen in laboratory animals might not indicate reduced sensitivity to aversive stimuli but a difference in response quality. This may impact their sense of control and predictability in their environment and consequently their well-being.

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LASA guidance on rehoming laboratory dogs

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Summary

A number of establishments have successfully rehomed laboratory dogs over many years – an exercise that has proved beneficial to the individual animal, the new owner and the staff at the rehoming establishment itself. However, experience had shown this is not always an easy task and certainly not one to be undertaken lightly if it is to be unequivocally beneficial to the individual animals concerned. The procedures adopted must be designed to ensure the well being of the animals and under no circumstances should their welfare be compromised. LASA has recently produced guidance notes, together with relevant background information to facilitate the rehoming process. This paper provides a brief overview of the guidance and the background information on which it is based. The guidelines refer specifically to dogs but the principles could be applied to rehoming any species used in the laboratory.

Introduction

This paper provides an introduction to the background and content of new guidance from the Laboratory Animal Science Association (LASA)[1] on the rehoming of laboratory dogs. The guidance has been developed in consultation with personnel from establishments that have homed a variety of laboratory species including dogs, horses, chickens, rabbits, sheep and rats, and who were enthusiastic about developing this further. The Royal Society for Prevention of Cruelty to Animals (RSPCA) in the UK, which rehomes thousands of companion animals each year, was also closely involved since the Society was interested in developing the concept of rehoming for laboratory animals as an alternative to euthanasia. Experts in dog behaviour also provided valuable input.

The guidelines have been developed by a LASA working party, following on from an initial workshop, which considered the options for post-experimental, breeding or surplus animals. The key question at the Workshop was whether euthanasia or re-use are always the only options when an experiment does not require the death of the animal, or whether more animals could be rehomed. The factors that affect these decisions, for example: the species and numbers that potentially could be rehomed; the legal controls; the veterinary perspectives; and the practical principles including issues for animals, the owners and the establishment, were carefully examined, alongside several case studies of successful rehoming initiatives in the UK, Europe and the USA.

Costs and benefits of rehoming

Rehoming has potential costs and potential benefits. If it is accepted that animal life is in itself important and it is possible to place an animal in a good home, then there are clear benefits for the individual animals concerned. There are also benefits for the staff, who feel they are doing something additional for the animals in their care. The establishment can also benefit as it can be good for public relations, demonstrating responsibility, care and concern for the animals it uses.

There are, of course, potential costs. Changes in the environment and in canine and human companions can lead to behavioural problems for the animals (although these can usually be resolved), and there may be problems for the establishment with respect to the time and resources required. There is also the potential for negative publicity.

Participants in the LASA workshop and subsequent working party agreed that the costs are outweighed by the benefits, and that rehoming is therefore a 'good thing to do'. The potential should, therefore, always be explored. The proviso is that it must be done properly to ensure that the well being of the animals is not compromised.

A rehoming framework

LASA concluded that rehoming must be done within a clearly defined framework, which allows comprehensive assessment of all the costs and benefits on a case-by-case, animal-by-animal basis. The LASA guidance provides the basis for developing such a framework. This could be set up under the auspices of local ethical review processes, ethics and animal care committees, with input from laboratory veterinarians and animal technicians being essential. The factors to consider are:

- any legal controls
- selection of suitable animals (e.g. considering health, temperament, experience)
- preparation of animals for their new environment
- assessment of the suitability of new homes and owners
- provision of advice to new owners
- working through third party animal welfare organisations
- follow up after rehoming

The guidance provides advice on each of these points, with further information available in an appendix where some interesting case histories are also presented. Three of the practical aspects are expanded below but for full details it is essential to read the complete report (LASA, 2004).

Preparation of animals

The laboratory environment is very different from a companion animal environment and successful rehoming depends on how well the dogs are prepared for the change. There are two key issues: firstly, veterinary care - animals will need to be vaccinated and wormed prior to release, and there needs to be an agreed policy on dealing with zoonoses such as *Campylobacter*; secondly, their mental preparation.

Dogs in a companion animal environment will be exposed to a huge range of new visual, tactile and auditory experiences, with a big change too in the humans and other dogs they encounter. This can lead to behavioural problems if not addressed properly. Developing in-house socialisation programmes for dogs and staff, habituating them to a range of sights and sounds, and training them to respond to basic commands and to walk on a lead will all help the dogs cope with the changes. Such activities have the additional

advantage of reducing stress associated with experiments. UK establishments have recognised the importance of this and are already developing socialisation programmes for their laboratory dogs as a refinement, regardless of the likelihood of the animals being rehomed. The most recent Report of the British Veterinary Association, Animal Welfare Foundation, Fund for the Replacement of Animals in Medical Experiments, Royal Society for the Prevention of Cruelty to Animals, Universities Federation for Animal Welfare (BVA/AWF/FRAME/RSPCA/UFAW) Joint Working Group on Refinement (Prescott et al, 2004) addresses dog husbandry and care, providing additional detailed information about dog behaviour, socialisation, habituation and training which is directly relevant to rehoming.

Assessment of homes and owners

Potential owners need to be committed to rehoming with realistic expectations of the dogs that they obtain. They need to be receptive to advice, and understand the nature of, and actually *want, a beagle.* They should not just be motivated by wanting an ex-laboratory dog. They also need to be willing and able to deal with difficult behaviour and cope with any negative public perception about owning an ex-laboratory animal. It has also been found that dogs re-home better in pairs, or to a home, which already has another dog. A quiet environment is preferable, and, given the nature of beagles, it needs to be escape proof!

Working with animal welfare organisations

Some animal welfare organisations have a great deal of experience in rehoming animals and it can therefore be very advantageous to work together with them. Rehoming can be done directly, i.e. direct to the new home with the animal welfare organisation acting as facilitator, as has happened in Germany, or indirectly in that the dog is first taken in by the animal welfare organisation and then homed onwards from there. This is the method that the UK RSPCA has used to rehome beagles from both a pharmaceutical company and a university. Either way it will be necessary to sort out responsibilities, for example for the cost of vaccination, worming, and neutering.

It is important, however, to recognise that there may be difficult public perception issues for both parties to deal with, and to communicate openly about these.

A successful conclusion

By following up on the progress of the rehoming process, an establishment shows interest and commitment and allows people (either establishment staff or the new owners) to discuss any concerns that they may have. It provides feedback to develop the programme and helps make decisions regarding whether to try another home if animals fail to settle. One example of recent feedback clearly illustrates how successful rehoming can be. Forty eight stock beagles were rehomed from a UK breeding company and generated the following quotes come from just some of the new owners:

"Good with the other dogs and with the children, sleeps a lot"
"House trained, good with the kids, still slightly wary of other dogs but good on lead"
"Loyal and affectionate, I wouldn't change him for the world"
"Good with children and lead walking, a bit wary of cars"
"Loving, no aggression, but objects to being left alone"
"The best cab - mate my husband has had in his long distance lorry!"

Clearly, very satisfied owners and happy dogs!

Acknowledgements

LASA would like to thank all the many individuals, establishments and organisations who have contributed to the LASA workshop, working group and the preparation of the full guidance.

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Biosafety and Risk Assessment

Non human primates handling in the BSL-4 laboratory 'Jean-Mérieux, in Lyon-France

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The BSL4 laboratory named 'Jean-Mérieux' was built by the 'Fondation Mérieux' in Lyon and inaugurated at the end of the year 1999. This laboratory was devoted to the study of biosafety level 4 microrganisms (P4 agents), belonging to different viruses families. These viruses are responsible for a high public health problems in the different countries where they are endemic but are the subject of a high interest from their potential use as biological weapons. Since january 2004, the 'P4 Jean-Mérieux' is a national laboratory under the responsability of INSERM. According a convention, the scientists of the Institut Pasteur unit of Biology of Emerging Viral Infections help to some technical aspects in particular the development of in vivo studies. The scientific objectives of the laboratory are to developp research programmes adressing diseases caused by P4 agents. The concept of the BSL4 in Lyon has benefited from recent advances in equipment and systems of biotechnology from the pharmaceutical and nuclear industries, following assurance and control quality to improve the high standard required for biosafety. Biosafety is important for high security laboratories, to protect the scientists from infection within the laboratory and to protect the environment from microrganisms handled in the BSL4. An area of this laboratory is devoted to the animal facility. This is the only BSL4 in Europe providing an animal facility for monkeys. This such confinment level lead to specific handlings of the animals, implying first a team of well trained persons to care about the non human primates. All experimental protocols are reviewed by the Regional Ethical Committe, for the well being animals. The animals are under constant video surveillance and could be followed by telemetry for clinical parameters. All blood analyses were done inside the laboratory on automatons. The first experimental protocol with infected monkeys was carried out in november 2002. It concerned the Lassa fever infection, wich is the main scientific research program of the unit. Studies on monkeys should be provide extremely valuable information for the development of prophylaxis and better adapted treatment against these expanding emerging diseases, caused by P4 agents.

Prions : safety working conditions and current legislation

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Prions are non conventional infectious agents causing the so called Prion Diseases or Transmissible Spongiform Encephalopathies (TSEs). Since the mad cow disease a great concern has been raised upon this field and numerous resources have been directed towards research on such pathologies. Prions are proteinaceous particles capable of transmitting a conformational change to their host-encoded cellular counterpart. Such pathological form of prions shows an enhanced resistance to the usual disinfection procedures. Moreover the fact that neither efficient treatment nor prophylaxis exist up to date implies that special security measures must be taken when exposure to prions occurs during experimental work. Notwithstanding the particular features of prions the hazard they represent is relative as, for instance, aerogenous transmission does not exist. The use of experimental animal models has gained an important role in the investigation of such diseases: transgenic models challenged with prions, lesion profiling protocols to differentiate strains or basic pathogenesis studies are good examples of it. The OIE has classified prions into the Disease List B: Transmissible diseases that are considered to be of socio-economic and/or public health importance within countries and that are significant in the international trade of animals and animal products. The legislative frame for experimental work with this kind of agents can be found in the EU Directive 2000/54/CE regulating the classification of biological agents as well as the safety measures to be taken including containment measures for laboratories and animal house facilities, individual protection measures, etc.

Balanced management of a pathogen free animal unit and experimentation with human pathogens

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The animal experimentation is today confronted with many scientific, regulatory and technology challenges, which will be reviewed. Breeding under specific pathogen (SPF) conditions protects mice from environmental pathogens which is achieved by air filtration and positive pressure of the mouse habitat This provides a protected environment allowing performing controlled experiments with defined variables such as a exposure to drugs, chemicals or even microbes. This defined environment allows a better interpretation of the effects of a single agent, although a real life exposure for both mice and mice represents a combination of several exposures, and the unwanted noise may hide the effect of the intervention. Therefore, the barrier with positive pressure and controlled atmospheric condition together with appropriate handling avoiding stress allows investigations of a given test procedure in mice. Most intriguing are infectious agents such as parasites, helicobacter and helminth co-infection, which are often clinically unapparent, but may make interpretation of eg immune responses impossible. However, what if the microbes are human pathogens such as Mycobacteria tuberculosis, the causative agent of tuberculosis? Here, in addition the investigator performing an infectious protocol needs to be protected from the infectious agents. In such situation a negative pressure condition with additional protection with a mask in a BSL3 facility is required. Therefore, the constraints to perform experiments providing meaningful results are complex and will be discussed in the context of the technical possibilities of the animal facility, the regulatory aspect and the scientific question addressed for each experiment. Importantly, if alternative, meaningful in vitro alternatives exist, the *in vivo* experimentation should be replaced or reduced to a minimum.

Containment testing of negative pressure isolators used to house laboratory animals infected with BL3 agents

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Summary

Animal husbandry isolators require stringent validation tests of protective efficacy before they can be used for containment of infectious agents. Three types of commercially available isolator, flexible film isolators (FFI), flexible half suit isolators (FHSI) and rigid half suit isolators (RHSI) designed to house animals infected with BL3 agents were subjected to both physical and biological bio containment testing. Physical testing performed on these isolators demonstrated that modifications were required to most of the isolators before they could be considered safe to use as containment systems. Microbiological tests have been applied to measure the degree of containment provided by the isolators and to correlate these results with physical tests to define future standards. The biological testing showed that when used with standard operating procedures, all the isolators provided an adequate performance (operator protection factor >105) once they had passed physical testing. The RHSI was found to perform to a high standard but was affected by the extreme pressure fluctuations caused by entry and exit from the half suit. The RHSI also was found to pressurise to a significant extent when compressed air was introduced. The FFI required stringent standard operating protocols involving the use of disinfectants to be followed to ensure an adequate protection factor during many procedures. The FHSI was found to be the isolator that gave the best overall containment performance. Future studies will investigate the effect of leaks and other accidents on the performance of these isolators.

Introduction

Animal models play a major role in the elucidation of pathogenic mechanisms of human infectious diseases and in the development of effective vaccines and therapeutics (Zak and Sande, 1999). In these models laboratory animals are frequently infected with high titres of pathogenic agents. It is a regulatory requirement to contain animals infected with BL3 agents (only those infective by the airborne route) during infection and subsequent housing to prevent exposure of laboratory workers to the agent (European Directive 2000, Advisory Committee on Dangerous Pathogens 1995, 1997). Standard laboratory containment equipment such as microbiological safety cabinets is not suitable for the housing of laboratory animals due to animal welfare concerns such as noise, space and vibration (Anon 1989). Therefore specialised equipment needs to be developed to meet both health and safety regulation and animal welfare requirements. In the UK these requirements are detailed in the guidance "Working safely with laboratory animals" (Advisory Committee on Dangerous Pathogens 1997). Attempts have been made to meet these requirements by the use of equipment such as individually ventilated cages (Hoglund and Renstrom 2001) and negative pressure isolators. A survey of animal laboratories in the UK working with BL3 agents showed that, in the majority of laboratories visited, negative pressure isolators were being used to contain rodents such as mice and guinea pigs. The three types of animal husbandry systems used are as follows:

1. Flexible Film Isolators (FFI)
2. Half Suit Flexible Film isolator (HSFI)
3. Rigid Half Suit Isolator (RHSI)

Flexible film isolators were introduced into the UK in the 1960s by Trexler and were originally designed to provide an economical way of deriving and maintaining germ-free animals that were originally kept in expensive stainless steel systems (Gustaffson, 1948, Trexler and Reynolds, 1957). These positive pressure isolators were further developed to protect immunosuppressed patients and even farm animals (Wilson et al 1973, Dennis et al 1976). Isolators were later developed to operate under negative pressure to house infected animals or for the transportation and nursing of patients suspected of harbouring dangerous pathogens (Harper et al 1983)

Most isolators manufactured for use in animal husbandry are constructed to operate at positive pressure to protect animals from infection from the external environment and their handlers. The requirements of a negative pressure containment isolator are very different to those of positive pressure isolators and test procedures for positive pressure isolators are not directly applicable to containment isolators (Lee and Midcalf 1994). It was therefore decided to investigate techniques to measure physical and biological manifestations of containment of negative pressure isolators.

At first each isolator type was tested using physical testing methods. Physical testing methods are essential in ensuring that basic standards are met but cannot be used to test how isolators performed when in use. It is key in evaluating protection that systems are tested under conditions of use. The most effective way to test complete operating systems is to use biological markers.

The physical testing of isolators involved filter testing, pressure hold testing, flow measurement and leak detection. If these tests were failed then repairs were made and the isolator was re-tested until it passed. Normal pressure fluctuations caused during operations were also measured. Biological tests were undertaken on one example of each isolator once the basic physical criteria had been met and involved filling isolators with an aerosol of a spore tracer and measuring the level of leakage from the isolator. In the biological test the ratio between internal and external concentrations provided a measure of the operator protection factor (OPF). The OPF is generally accepted as an indication of containment performance. An OPF of greater than 105 is generally accepted as an adequate containment performance (Advisory Committee on Dangerous Pathogens 1995).

The aim of the study was to determine the containment performance of each of these units and to determine which of the isolator design features influence the OPF. In addition data was obtained that compared and correlated the results

of physical and the biological testing, allowing physical test standards to act as an indicator of an acceptable biological performance.

Isolator procedures

As protocols of usage for each isolator varied according to the day-to-day tasks required in animal husbandry e.g. transfer of animals, waste removal and supply of materials, these procedures are described separately for each isolator.

Flexible Film Isolator (FFI) System

This system was used for short-term housing (ca7 days) of guinea pigs infected with a hazard group 3 bacterial agent, Bacillus anthracis. An outline of the procedures undertaken within the FFI and how it interacts with the other parts of the containment system are shown in Figure 3. Challenge with the pathogen was by the sub-cutaneous route and was conducted in a separate adjacent room on a double HEPA filtered re-circulating downdraft table (Astec Microflow) by staff wearing positive pressure RPE. Challenged animals were then passed through a transfer port in the wall to a holding isolator and transfer isolators were then used to move the animals to the housing isolators. When samples or waste were removed from the isolator, the bags were moved to the transfer port where they were sprayed with 5% sodium hypochlorite and left for 30 minutes before removal. Similar hold times were used for transfer to transfer isolators. During transfers the isolators were attached using a plastic sleeve held onto each isolator by rubber bands, metal clips and tape and the sleeve was decontaminated as above after the transfer.

Flexible Film Half Suit Isolator (FHSI)

The FHSI was used for longer term housing (4-17 weeks generally but potentially up to a year) of guinea pigs challenged by the aerosol route with Mycobacterium tuberculosis. An outline of the procedures undertaken within the FHSI and how it interacts with the other parts of the containment system is shown in Figure 4. The guinea pigs were infected on a downdraft table, by nose only aerosol exposure, in a separate adjacent room and passed through a hatch into transfer isolators which were used to load the isolators. The transfers were carried out as for the FFI but in this case the validated holding time was 10 minutes. Once loaded the isolator was provided with consumables through the transfer port, again using a 10 minute hold time. Waste was removed through the waste port located in the floor of the isolator. A long tube of durable plastic was attached to the outside of the waste port using heavy duty tape. The distal end of the tube was closed by double cable ties around a swan neck. The tube served to receive waste material that had been previously bagged inside the isolator. The outside surfaces of waste bags were sprayed with 5% Hycolin; the cover of the waste port was opened and the waste bag was then placed in the tube and sprayed again with the Hycolin solution and the waste port was replaced. After a ten minutes period the waste bag was moved to the far end of the tube where it was isolated by two cable ties. This double bagged segment was released by cutting between the cable ties and then removing for autoclaving.

Rigid Half Suit Isolator (RHSI)

The RHSI was used to house and carry out procedures on mice and therefore no subsidiary transfer isolators or other equipment were required. Material leaving the isolator would do so through a ventilated pass box or a dunk tank filled with 5% Tegodor. An outline of the procedures undertaken within the isolator is shown in Figure 5.

Physical testing of the Isolators

Dispersed Oil Particle (DOP) filter testing

Dispersed oil particles (Ondina EL, Shell) were generated using a cold smoke generator (Phoenix Instruments SG30/ SG20). These particles were used to challenge the face of all filters individually either directly onto the filter face or through ports between the double HEPA filters. The average particle size of the aerosol produced by the generator is 0.3 microns. A Phoenix JM7000 photometer, with a pistol shaped sampling nozzle to scan the filter face, was used to measure the penetration of DOP through the filters. In the testing of the FFI, a housing was attached to the inlet filters to allow even challenging of each inlet filter with smoke. The penetration of smoke was calculated as a percentage of the challenge concentration. A penetration of 0.003% or less was regarded as acceptable.

Positive pressure hold testing and leak testing

Positive pressure hold testing was carried out on the flexible isolators to assess the strength and leak-tightness of the canopy. This was carried out at static environmental conditions by blocking all the supply and extract ducting and pressurising the isolators with compressed air to 200Pa in the case of the FFI and 150 Pa in the case of the FHSI. An Airflow Developments PVM-100 digital manometer was used to measure the pressure differentials during the pressure hold testing. The values used were on the manufacturer's recommendation and followed at least 30 minutes of pre-stretching at 250 Pa. The pressure was then monitored for 30 minutes and if the pressure loss was less that 10% then the test was passed. If not the isolator was filled with DOP at positive pressure and the photometer probe was used to scan all areas where leaks may occur. The transfer hatch doors were examined independently. Any minor leaks found were treated with sealant and the pressure hold test repeated until successful.

Measurement of air flow rates

The air change rate was calculated by measuring the volumetric inflow (or extract) using a vane anemometer. In the case of the FHSI and the FFI the flow was measured at one point by taping the head of the anemometer to the inlet ducting in such a way that all the flow into the isolator went through the head. With the RHSI the flow was measured at five points over the face of the extract filter, an average taken which was multiplied by the cross sectional area of the filter. The air change rate was determined using the following formula:

The volume of the isolator was taken as the volume inside the metal frame.

$$\text{Air change rate } (h^{-1}) = \frac{\text{Volumetric inflow } (m^3\ min^{-1}) \times 60}{\text{Volume of isolator } (m^3)}$$

Pressure Measurement

The initial pressure differential of the isolators was measured and adjusted to the level agreed with the manufacturer. Pressure fluctuations were measured using the manometer to record the pressure differential every two seconds while the operator used the isolator either by vigorously entering and exiting the half suits or by moving in

and out of the sleeves of the isolator.

Microbiological testing

A spore suspension (3 x 109 per ml) of aero-stable Bacillus subtilis var niger was generated from Collison 3 or 6-jet nebulisers (May 1973), operating for two minutes, within the isolator beside the cage racks. An all glass impinger (AGI), operating at 11l/min (May 1957), containing 10ml of sterile distilled water, was operated for 2 minutes to measure the aerosol concentration within containment. The concentration outside the isolator was measured by two cyclone samplers, operating at ca700 litre min-1 and using sterile distilled water as a collecting fluid and by either one in the FFI tests or two 30 litre min-1 Casella slit samplers containing Tryptone Soya Broth agar (TSBA) plates. These samplers were operated for five minutes.

The collection fluid from the cyclone and AGI samplers was diluted and plated out on Tryptone Soya Broth agar (TSBA) plates. All the TSBA plates were incubated for 24 hours at 37oC before being counted.

Experimental Design

Testing was carried out to determine the protection factor afforded to operators whilst carrying out standard procedures. The concentration of aerosol within the isolator was divided by that measured outside to give the operator protection factor. If the OPF was greater than 105 then the performance was regarded as adequate. Since the isolators were of different designs it was difficult to match exactly the tests between the isolators. Only a few tests like static operation were exactly the same for each system.

Normal Use of Isolators

A series of experiments were carried out to assess how the isolator performed when carrying out normal procedures. The following procedures were studied.

1. Normal use of isolator – with and without operators. Isolator used with an operator entering and using the half suit (or gloves in the case of the FFI) in a careless fashion.
2. Waste Removal (FFI and FHSI only) – using the procedures as described above using an appropriate sporicidal disinfectant. (5% sodium hypochlorite)
3. Transfer of Material (FFI and HFSI only) – Transfer of material from isolator to transfer isolator for both isolators. Transfer from the adjacent room to isolator in case of FHSI.

Results

Physical Test Results

The results of the initial physical testing of all the isolators are shown in Table 2. The pressure fluctuation data is shown in Table 3 and Figure 6.

Microbiological test results

The results of the microbiological testing are shown in Tables 4-6. In all but one instance OPFs were obtained which were greater than 105. The results for the RHSI are complicated as the use of the Collison nebulisers within the isolator caused significant losses in negative pressure from 70 to 30 Pa in one case and –25 Pa to + 5 Pa which led to the reduction in the OPF to below 105. This phenomenon was not noted for the other two isolators.

Discussion

Physical test results

All the isolators were tested to the same basic standard using conventional testing methodologies and were shown to pass these tests (Table 2). All the flexible isolators bar one were shown to be able to hold positive pressure for 30 minutes with less than 10% loss. The other isolator had a defective canopy. All the filters were shown to perform to the expected standard. However, to achieve this performance a great deal of commissioning work had taken place. The positive pressure DOP testing often located leaks in seals or gaskets that required repair with silicone sealant before positive pressure testing was successful. A small but significant percentage of the original HEPA filters were shown to fail and had to be replaced. This shows the importance of careful construction, commissioning and testing of flexible film isolators.

The regular physical testing of laboratory ventilation systems is a requirement of UK health and safety legislation. Isolators require a higher degree of testing and maintenance than laboratory containment equipment to ensure they pass this testing. This is due to the materials of construction being less resilient than those used in safety cabinets. Therefore the UK recommendations for regular six monthly testing of this equipment if used for handling BL3 agent infected animals should be adhered to.

The measurement of the pressure fluctuations during the use of the three isolators gave very different results for each unit (Table 3). When one operator used the FFI in a vigorous fashion the maximum fluctuation was 59 Pa while when two operated the fluctuation was 84 Pa. However, on neither occasion was the isolator found to reach positive pressure. The FHSI generated a very small pressure fluctuation (16 Pa) when the suit was entered and exited as is shown in Figure 7. The pressure fluctuation caused by entering and exiting the RHSI was almost a degree of magnitude higher than the FHSI when measured at two different working static negative pressure differentials, 34 and 75 Pa giving fluctuations of 156 and 154 Pa, respectively. When operated at the lower pressure differential the isolator reached a positive pressure differential of 55 Pa. The increase of static pressure differential to 75 Pa was recommended to the isolator users.

The main reason for the greater pressure fluctuations of the RHSI as compared to the FHSI was the ability of the FHSI canopy to absorb the pressure increase caused by the entry into the half suit and the pressure decrease on exit. The very large panel filters used in the FHSI also allowed rapid absorption of any pressure increase. However, it is likely that the use of both supply and extract fans in the RHSI slowed down the isolator response to the pressure differences. The FFI pressure fluctuations are higher than those for the FHSI due to the significantly smaller volume of the isolator and the presence of cages racks completely covering one of the longer walls giving a far lower amount of flexible canopy available to expand and contract. Another reason for the poorer performance of both these isolators was the narrow ducting connecting both the supply and extract filters. A problem with the use of isolators with large pressure fluctuations is the difficulty in using pressure alarms to indicate pressure loss. These alarms would regularly sound during use of the RHSI and FFI unless alarm systems which only sound when the pressure goes out of range for a significant length of time were used. Isolators should be used at pressure differentials that prevent them going positive under any possible condition

of use. If possible the use of supply fans should be avoided as extract fans alone cope with pressure fluctuations to a greater degree.

During the biological testing it was found that when Collison nebulisers were operated in the RHSI the degree of negative pressure within the isolator dropped significantly by 30 to 40 Pa due to the provision of a small amount of excess air (ca10l/min) at high pressure. (This was not found with the flexible isolators.) Therefore, when the RHSI was operated normally at –25 Pa it operated at + 5 Pa with the Collison nebulisers switched on. When the isolator negative pressure was increased to 70 Pa it fell to 30 Pa with the Collisons operated. This phenomenon seems to have been due to the influence of the supply fan in reducing the response of the extract fan to pressure and air supply fluctuations.

Biological testing

Three different types of isolators have been subjected to a range of rigorous biological tests while being used to perform simulated routine tasks. When all the isolators are operated according to their standard procedures the OPF obtained always exceeded 105 and exceeded 106 for all procedures in all isolators except when the RHSI went positive. (Tables 4-6). In most cases the aerosol levels outside containment were below background levels. Therefore, if these isolators were correctly used they should always give an acceptable performance (OPF >105) for containing animals infected with BL3 agents. This shows that once the physical test standard had been met an acceptable biological test standard was also achieved as long as procedures were followed. However, to achieve this standard the procedures used in the FFI were dependent upon the heavy use of disinfectant sprays and long hold times (30 minutes). The safe use of this isolator required careful and precise use of standard operating procedures. The FHSI isolator required a shorter disinfection step lasting ten minutes even though the same organism was used. The RHSI had a built in dunk tank and ventilated pass box which precluded use of a disinfection step.

The RHSI biological tests were complicated by the magnitude of negative pressure within the isolator reducing during operation of the Collison nebulisers. When the Collisons were used to test OPF during entry and exit of the unit an initial pressure of –25Pa rapidly increased to + 5 Pa and microbial aerosol was released. This demonstrated the containment ability of the unit under positive pressure. The fact that the isolator was not completely leak tight was demonstrated by the measured OPF of 5.07 x 104 (Table 10). However once the negative pressure was increased to 70 Pa, the negative pressure with the Collisons operating was 30Pa and the OPF improved to above 106. However, the larger the negative pressure in the isolator the more difficult it can be to work in half suits.

The three types of isolators commonly used in the UK each passed stringent physical testing after a period of commissioning and repair. After this testing and modification had been undertaken each isolator was shown to be capable of giving an acceptable degree of protection as shown by biological testing when operated according to standard operating procedures. This indicates that isolators that pass the physical testing will also give a good biological test performance.

The choice of isolator will depend on what animals are used and what procedures are undertaken within them and on commercial, welfare, ergonomic and safety reasons. This study has shown that isolators can give a good level of containment if used correctly. In future studies it is intended to investigate in more detail how these physical factors can be manipulated in order to improve the containment performance of isolators and how isolators perform under accident scenarios.

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Table 1. Physical Features of Isolators Tested

Isolator	Animals Housed	Re-circulating or Thimbled	Volume (m^3)	Fans	Pass Box	Transfer Isolator Required	Alarms	Special Features
FFI	Guinea Pigs	Re-circulating	2.4	Extract	Non-ventilated	Yes	Pressure, Power	
FHSI	Guinea Pigs	Re-circulating	4.7	Extract	Non-ventilated	Yes	Pressure, Power, Airflow	Separate Waste Port
RHSI	Mice	Thimbled	4.1	Extract and Supply	Ventilated	No	Fan Power	Dunk Tank

Table 2. Physical Testing Results of the Four Isolators

Isolator	No Isolators Tested	Average ach (s.d.)	Working Pressure Differential (Pa) (s.d)	Pressure Tested (Pa)	% Pressure Loss Within 30 minutes (s.d)
FFI	13 (12)	15.56 (0.75)	42.3 (3.17)	200	7.07 (1.56)
FHSI	3	22.6 (0.60)	30.7 (0.58)	150	4.20 (1.01)
RHSI	1	41	75 (25)	ND	ND

s.d – standard deviation, Pa- Pascals

Table 3 Pressure Fluctuations Caused By Use of Isolators

Isolator	Procedure	Working Pressure (Pa)	Range of Pressure Fluctuation (Pa)	Maximum Pressure (Pa)	Minimum Pressure (Pa)
FFI	One Person working vigorously	-40	59	-19	-78
FFI	Two People working vigorously	-40	84	-1	-85
FHSI	One Person Exiting and Entering Half Suit	-36	16	-31	-47
RHSI	One Person Exiting and Entering Half Suit	-34	156	+55	-101
RHSI	One Person Exiting and Entering Half Suit	-75	154	-4	-158

Table 4. Operator Protection Factors Obtained During Use of Isolators

Isolator (Pa)	Procedure	N o Tests	Average Conc'n Inside Isolator (cfu/m^3)	Average Highest Conc'n Outside Isolator (cfu/m^3)	Operator Protection Factor
FFI (-40)	Vigorous use - sleeves	5	1.51 x 10^7	BDL < 10	>1.51 x 10^6
FHSI (-30)	Vigorous use - sleeves	2	1.62 x 10^8	BDL <10	>1.62 x 10^7
FHS I (-30)	Exit and entry from suit	3	1.15 x 10^8	BDL <10	>1.15 x 10^7
RHSI[a] (-70)	No activity	2	6.59 x 10^7	37.1	1.78 x 10^6
RHSI[a] (*-70)	Exit and entry from suit	5	1.48 x 10^8	78.6	1.89 x 10^6
RHSI[b] (-25)	Exit and entry from suit	3	1.85 x 10^8	3.65 x 10^3	5.07 x 10^4

[a] – negative pressure without Collisons operating -70Pa with Collisons operating –30Pa. BDL –below detection limit (10 cfu/m^3)
[b] – negative pressure without Collisons –25Pa, with Collisons operating +5Pa.

Table 5. Operator Protection Factors Obtained During Waste Removal From Isolators

Isolator	No Tests	Average Conc'n Inside Isolator (cfu/m^3)	Average Highest Conc'n Outside Isolator (cfu/m^3)	Operator Protection Factor
FFI	5	2.30 x 10^7	BDL <10	> 2.30 x 10^6
FHSI (1)	4	8.97 x 10^7	BDL <10	> 8.97 x 10^6
FHSI (2)	3	6.34 x 10^7	BDL <10	> 6.34 x 10^6

Table 6. Operator Protection Factors Obtained During Transfer of Material to Transfer Isolator

Isolator	Procedure	No Tests	Average Conc'n Inside Isolator (cfu/m^3)	Average Highest Conc'n Outside Isolator (cfu/m^3)	Operator Protection Factor
FFI	Docked Isolators	5	3.07 x 10^7	BDL <10	3.07 x 10^6
FFI	Transfer to Transfer Isolator	4	2.08 x 10^7	BDL <10	2.08 x 10^6
FHSI	Transfer to Transfer Isolator	3	1.01 x 10^8	BDL <10	1.01 x 10^7
FHSI	Transfer from other room	3	1.04 x 10^8	12.1	8.57 x 10^6

Figure 1. Flexible Film Isolator

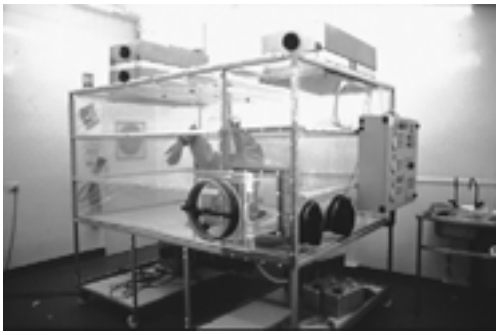
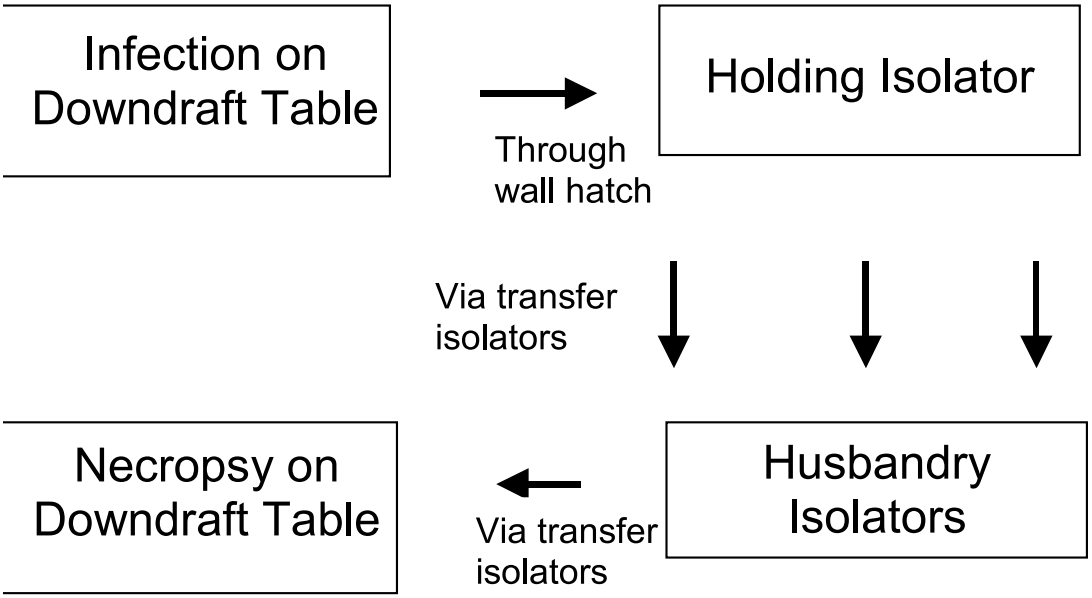
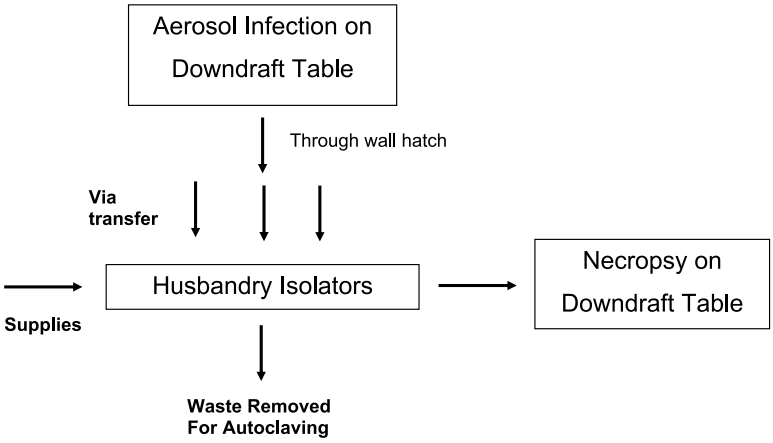
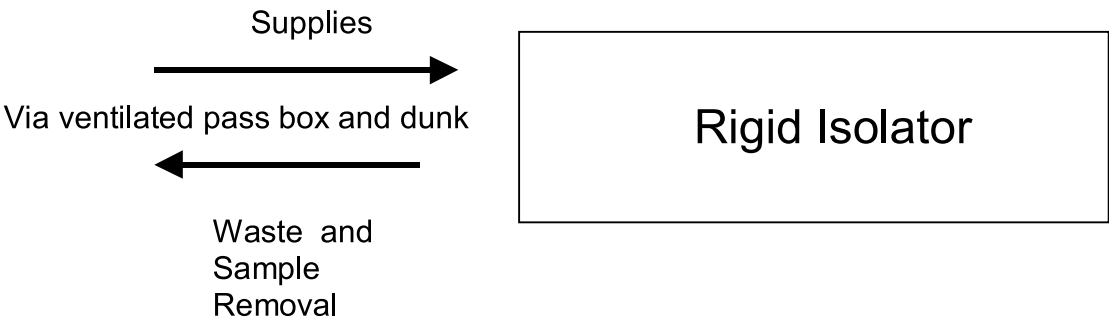
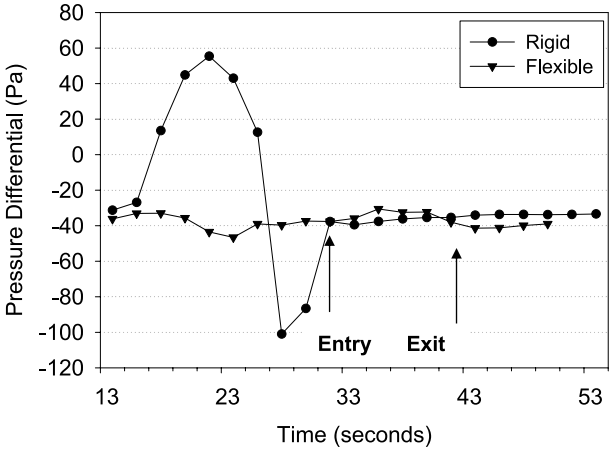


Figure2. Flexible Half Suit Isolator









Note: The x axis represent the seconds reading from the digital manometer. Total duration of experiment 40 seconds for RHSI, 36 seconds for FHSI

Balancing biosafety, research and animal welfare

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How can a biomedical research institution balance the needs of appropriate biosafety procedures and the welfare of the animals in order to meet the goals of the research? In this session we will review the regulatory requirements for recombinant DNA (rDNA) applications in modified stem cells, transgenic animals, and gene therapy research. We will then discuss these requirements through the review process by the Institutional Biosafety Committee (IBC) which reviews/approves the use of rDNA, the Animal Care and Use Committee (ACUC) which reviews/approves animal studies, and follow the research project within the animal facilities which completes the research project. The speakers serve as the veterinary members of the IBC, ACUC, manage the animal program and also provide collaborative research support. They will cover applicable requirements of the Center for Disease Control (CDC) manual on biosafety practices, the requirements of the NIH Recombinant DNA Guidelines, the peer review process of rDNA research by the IBC, the review of the animal study proposal by the ACUC and how these different groups relate to produce safe, humane and productive research. They will also discuss animal facility design issues related to biohazard containment and procedures.

Are clean rodents good models for Man?

Influence of commensal flora on the biological reactivity of laboratory rodents.

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Summary

Rodents, and in particular laboratory mice, have been used for decades as experimental animals. Many of the studies in which they were involved have allowed to more precisely describe their features. Parameters that characterize some of their biological functions, such as reproduction or metabolism, are becoming available for a number of strains. To guarantee stability of the animal models, updated recommendations are published concerning monitoring and control of the health status, the environment and their genetic identity. However, two years ago, observation of alterations of some immunological animal models revealed that a more extended definition of the biological reactivity of the laboratory rodents and of the parameters that may influence them might be required. In order to initiate a process leading to a better characterization of our animal models, a workshop was organized in 2003 at the Institut Pasteur, with the collaboration of the Institut National de la Recherche Agronomique (I.N.R.A.). This gave an opportunity to discuss the influence that current breeding procedures could have on the biological reactivity of laboratory rodents. A summary of the discussions that took place during this workshop follows.

Keywords: laboratory rodents, commensal flora, biological reactivity.

Three years ago, several research groups observed significant changes in their experimental animal models. At the Institut Pasteur, two types of alterations were noticed in SPF mice purchased from commercial sources, contamination of their respiratory tract and pulmonary tissue samples by opportunistic microorganisms and changes in some immunological responses in different experimental models. Similar observations had been noticed in other research centers, and previously reported.

Those alterations significantly impeded or stopped the development of funded programs to analyze immune responses induced by infectious agents or vaccines. Given these serious negative impacts, a workshop on this topic was co-organized by the Institut Pasteur and the I.N.R.A. in September 2003. The aim of this meeting was to bring together commercial breeders and users of laboratory rodents to discuss the factors that influence the biological reactivity of the animals under study.

Paola Minoprio opened the workshop with a summary of the observations that had been reported by groups from the Institut Pasteur since 2001. Representatives of 3 breeding companies (Patrick Hardy, Charles River Laboratories, Robert Leblanc, the Janvier breeding centre and Stephen Hillen, Harlan) presented their respective zootechnical and health control procedures. Since the observed alterations were exclusively affecting the immunological field of research, scientists that study the interactions between the environment and the immune system were also invited to present data concerning the influence of food, hygiene and micro flora. The meeting ended with a discussion to debated the respective roles and responsibilities that FELASA, managers of animal facilities and scientists should exert to define and guarantee the stability of the animal experimental models.

Presentation of the modifications of the experimental models

The first modifications of the biological reactivity of laboratory rodents were reported in June 2001 in the Institut Pasteur. Some scientists were then unable to reproduce some of their previously observed and published data. Two major types of modifications were noticed and were only affecting research groups in immunology. The protective barrier effect of mucosal surfaces and the immune function were altered. Inbred (C57BL/6, BALB/c, C3H/HeN) and outbred (OF1) strains were concerned. The impact on the research groups that were affected was serious since some of their programs that were linked to the development of vaccines or patents were subsequently interrupted.

Some opportunistic microorganisms were isolated from the lung of mice, thus revealing a major break of the so-called “barrier effect” normally provided by the respiratory mucosal flora. These contaminants were isolated after experimentally infecting the mice with influenza virus but also in non-immune animals. The biological responses towards experimental infections were also modified. A decreased susceptibility of BALB/c mice towards *Bordetella bronchiseptica* was reported. Finally, the immune responses induced by vaccinal protocols were also altered. For instance, in a murine model of Chagas disease, the previously described vaccinal protective effect of one protein produced by *Trypanosoma cruzi* against an infection by this parasite was lost.

In order to identify what caused these alterations, Paola Minoprio’s group further studied different biological features of these animals. The haematology revealed eosinophilia and leucocytosis. The immune system of non-immune/uninfected mice was also more precisely explored. A decrease in the number of cells that could be isolated from the spleen was reported. The function of the lymphocytes isolated from the mice was also altered. For instance, for a strain coming from a given breeder, the cellular responses to stimulation by common mitogens such as LPS or ConA were altered and serum IgG (immunoglobulin G) titres were significantly reduced in comparison to those reported several years before. As will be discussed further, these are observations that are typically reported when the microbial environmental

stimulation of the animals' immune system is very low.

Environmental factors influencing the immune system: the role of the commensal flora

Though the immune system is already functional in newborn mice, it never stops evolving after birth in order to adapt to the environment. So environmental stimulations do not always cause disease. On the contrary, they are mostly non-pathological but induce responses that create a first line of non-specific natural defence against opportunistic and pathogenic microorganisms. The gut commensal flora contributes to this constant non-pathogenic stimulation of its host by activating and modulating the immune system both at the intestinal and peripheral levels (for a review, see: Immune modulation by the intestinal microbiota by Marie-Christiane Moreau; in "Gastrointestinal Microbiology", Arthur Ouwehand and Elaine Vaughan (Eds), Marcel Dekker, INC, NY. in press).

The natural antibodies (Abs), and in particular those of the IgA isotype that are secreted in the intestinal lumen, contribute to this defence process. Germ-free mice are good models to study the effect of the flora on the immune system. Intestinal secretory IgA levels are non significant in germ-free mice. The implantation of a commensal flora in these animals induces an increase in the number of IgA producing cells and the development of intraepithelial lymphocytes at the intestinal epithelium level. In the gut, the commensal flora not only influences the innate immunity but also the specific immune responses. In order to preserve its integrity, any mammalian organism needs to continuously distinguish exogenous antigens (Ags) that are potentially pathogenic, from those that don't represent any danger. At the gut level, mechanisms of oral tolerance repress immune reactions that could be triggered against food Ags. Oral tolerance can be broken in mice by the injection of bacterial toxins (cholera toxin or toxin from E. Coli.). Breaking oral tolerance is far easier to obtain in germ-free mice than in mice carrying a bacterial flora, indicating that the commensal flora regulates the oral tolerance process. Moreover, some bacterial populations present in the flora, such as Bifidobacteria, provide a better resistance than others towards intestinal infections such as salmonellosis. Finally, the gut commensal flora also influences systemic immune responses. Thus, implantation of a flora in germ-free mice induces an increase in the serum IgG levels. Immune responses towards non-digestive infectious agents such as in experimental cutaneous leishmaniasis are also modified in germ-free mice.

The influence of the flora on the biological reactivity of its host depends both upon its composition and the way it gets implanted in the gut. For instance, the study of some experimental models such as induced-arthritis has shown that Bifidobacteria can exert opposite immunomodulating effects to those of Bacteroides. The presence of one or both of these bacteria in the flora will thus influence the development of the arthritis and possibly the outcome of other inflammatory and infectious processes. Besides, the implantation of the gut flora needs to occur right after birth in order to trigger an optimal effect on the immune system. Thus, studies on caesarean-born or premature babies have demonstrated that a delayed colonization of the gut with a limited number of bacterial species tends to be pathogenic.

Potential causes of the alterations reported in 2001: the hygiene hypothesis

The preliminary data from Paola Minoprio's group and the different presentations given by the speakers that were invited during the 2003 workshop supported the hypothesis that the decrease in the biodiversity of the rodents' commensal flora has caused the alterations that appeared in 2001. Indeed, the efficiency of the mucosal barrier effect and the levels of serum IgG, which were both reported to be altered, are dependent upon and correlated to the diversity of the bacterial populations found in the gut. Since no modification of the implantation flora used by laboratory animal breeders has been performed in 2000-2001, it is more likely that variations in some environmental factors influencing the composition of the flora might be indirectly responsible for the observed alterations. In adulthood, the composition of the gut flora is influenced by different unstable parameters such as the food and the microbial composition of the environment.

Food is a substrate for the flora and can thus promote or inhibit the growth of the different bacterial species that colonize the gut. The texture of food also influences the intestinal transit and thus determines the duration of contact between the bacteria and their substrate. Moreover, the microorganisms that are present in the food directly interact with the intestinal immune system. Indeed, though sterile, the food still contains bacterial walls that can stimulate cells of the innate immunity via receptors present on their membrane that recognize specific patterns on those bacterial walls. Nevertheless, the tests ruled out this hypothesis.

The composition of the gut commensal flora could also vary depending on the hygiene level of the environment. Whereas the flora that is implanted in newborns mainly comes from their mother, non-pathogenic microorganisms originating from the environment later enrich it. Evidence has shown that birth by caesar prevents the natural colonization of the gut and that, though the improvement of the hygiene level in hospitals has favoured a reduction in infections during the last century, it is nevertheless detrimental for optimal implantation of commensal flora in newborns. In humans, separation of the premature newborn from their mothers will also delay the colonization of their gut by commensal bacteria whereas breastfeeding will favour bacterial exchanges between mother and child. Antibiotics given to the mothers will also influence the composition of the flora that they can transmit to their newborns. Thus, the increase in the hygiene level of the environment will alter the animal immune reactivity, because of the modifications of the implantation process and of the composition of the flora that it will induce.

Therefore, we suggest that the degeneration of the non-pathogenic microbial environment of the laboratory rodents contributed to induce the alterations of the animal experimental models that have been reported since 2001. During the last decade, the improvement of the hygiene level of the commercial breeding units has indeed reduced the possibility of bacterial drift of the rodents' gut commensal flora, and thus limited the sources of non-pathogenic stimulation of their immune system. An argument in favour of this hypothesis is the fact that the modifications of biological reactivity of the rodents were reported after the simultaneous renewal of the production units of the commercial breeders. Finally, on the basis of epidemiological studies, it has been suggested that the improvement of the living conditions and especially the modifications of the composition of the gut commensal flora and the decrease in the incidence

of infectious diseases have contributed to the observed increase in the number of allergic patients in developed countries. Since laboratory rodents have benefited from a recent and significant improvement of the hygiene level of their environment and of their health status, it would not be surprising that their biological reactivity would change in the same manner. Therefore, these animals would be good models to study the evolution of the immunological characteristics of human populations in developed countries.

Working procedures that favour an increase in the hygiene level of the breeding units

The three representatives of the breeding companies presented their technical and health control procedures. The high increase in the generation and trading of new genetically modified rodents at the end of last century required the improvement to the hygiene level of the breeding units. Some of these new strains are immunocompromised and cannot survive in a conventional microbial environment. Standard operating procedures for health status monitoring are followed in order to reduce the possible spread of pathogens in the production units and in the experimental units of the clients. Working procedures also involve the recycling of the production units, either following detection of opportunistic or pathogenic microorganisms, or performed at regular intervals to anticipate and avoid the occurrence of any contamination. During recycling, the production unit is emptied, cleaned and sterilized. The animals that are introduced in the recycled unit either come from another unit (Harlan), or by caesarean-derived animals carrying a known implantation flora (C.R.L.). C.R.L. developed their implantation flora from the one described by Schaedler in the 60's, to which they added several bacterial species in order to improve its mucosal barrier effect.

The breeding and health monitoring procedures adopted by commercial breeders closely follow the recommendations that are regularly published by FELASA. Together with a regularly updated list of pathogenic or interfering microorganisms, which are recommended to be monitored in the breeding and experimental units. Proper education and training of the professionals involved in laboratory animal breeding and accreditation of diagnostic laboratories will also contribute to reach this objective. However, these recommendations are only guidelines that must be adapted to each particular case. Indeed the obligations of a commercial breeder differ from those of an experimental facility. The impact of the listed pathogens is also different depending on the field of research that is concerned. The responsibility of the managers of those facilities is thus to adapt the FELASA recommendations to their own situation.

Conclusion

The stability of animal experimental models is required in any scientific research field. However, no organization has ever published any biological standards that laboratory rodents should satisfy. Values that can be considered as physiological for some biological parameters can be found in publications. Many of them are also available on the Jackson Laboratory web site. Nevertheless, because of the diversity of the fields of research, it will certainly be difficult to define such criteria for each particular animal experimental model. It is desirable that some specific markers are determined to detect possible drifts in the animal models. Since the biological reactivity of living organisms is highly influenced by the environment, the parameters that influence the biomarkers that will be defined should also be clearly identified and monitored. The 2003 workshop on commensal flora and biological reactivity of laboratory rodents focused on the influence that the non-pathogenic microbial environment exerts on the gut flora and the immune system of the animals. The current working procedures that are followed in commercial breeding units are required to satisfy health standard recommendations, but they also prevent the natural colonization of the gut with environmental microorganisms. Introducing new additional bacterial species in their implantation flora could still artificially perform enrichment of the rodents' microbiota. Moreover, molecular tools are already available to monitor the composition of the commensal flora and these could be adopted to monitor the drifts in the composition of the animals' flora. Finally, the same reasoning can be extended to other fields such as behavioural research where modifications of the environment and in particular its enrichment may also have important consequences on the already established experimental models.

The digestive microbiota: an important regulatory factor for host's immunity

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The intestine is the largest lymphoid organ in the body by virtue of the number of lymphocytes that it contains and the quantity of immunoglobulins that are produced by some of these lymphocytes. From birth to death, the gut is colonised by a diverse, complex and dynamic bacterial ecosystem that constitutes the digestive microbiota (DM). In new-borns, this flora develops sequentially. In growing mice, facultative anaerobes are first established, while later and according to dietary diversification, strictly anaerobic bacteria appear. The DM becomes more and more complex until the age of 6 weeks when it is considered to have reached its adult characteristics. In mice the DM is present in the stomach and in distal parts of the gut, ileum and mainly in the colon (10^{10}-10^{11} bacteria/g of content). It is believed that only predominant bacteria are able to exert a function (>10^{6} bacteria/g of content). The relationship between the DM and the intestinal immune system (IIS) can be considered as a 'symbiosis' . The IIS does not mount any immune response to get rid of the DM. In return, the DM profoundly influences the ISS as well as the peripheral immune system. The DM can thus activate, modulate and regulate immune responses. In some cases, the bacterial effects are induced by the whole DM whereas in others, only one predominant bacteria strain is responsible for a given immunostimulating effect. During the neonatal period, the role of the DM is believed to be of particular importance and to have many outcomes in later life. Most of the data concerning the relationship between the DM and the IIS have emerged from original experimental animal models of germ-free and gnotobiotic mice, i.e. germ-free mice colonised with known bacteria. The IIS generates two important immune functions:

The first one is a protective function. It is performed by cellular responses and secretory IgA antibodies (Abs) in order to protect the mucosa against pathogenic micro-organisms and translocation of commensal bacteria.
The second one is a suppressive function, also called oral tolerance. It is characterised by several regulatory mechanisms that aim at inhibiting local and peripheral immune responses toward harmless antigens (Ags) present in the intestine (e.g. dietary proteins and bacterial Ags of the DM).

When these functions are altered, diseases such as enteric and/or systemic infections, hypersensitivities to dietary proteins and inflammatory bowel diseases can develop. The presence of the DM has been shown to play a fundamental role in the development and the activation of the IIS, especially during the development of IgA secreting plasmocytes. These studies demonstrated the importance of the diversification of the DM on the completion of the development of the IIS in young mice. Other reports have shown that the composition of the dominant DM modulates the specific anti-rotavirus sIgA antibody response, by either enhancing or suppressing it. Other experimental data have revealed the important role played by a single bacterial strain present in the dominant DM, with respect to the set-up of some key regulatory processes involved in oral tolerance. Other results have brought to light the role of the DM on the peripheral immune system. Macrophages and dendritic cells (DCs) play a key role in the activation of the immune system. Their Ag-presenting activity and their ability to synthesize numerous pro-inflammatory chemiokines and cytokines allow them to modulate specific immune responses, and regulate immune processes such as the Th1/Th2 balance. Recent studies suggest that neonatal DCs become fully competent for these innate functions after being activated by bacterial stimuli afforded by the DM. Natural serum IgG and IgA Abs, which production is also influenced by the DM, have also been shown to play important regulatory roles on specific humoral immune responses, especially towards self-Ags. In mice, it has been demonstrated that they contribute to the development of the splenic B-cell repertoire. These examples as well as others, show the crucial roles exerted by the DM on the hostâ€™s immune responses. Today, the lack of a clear definition of the composition of the DM of laboratory rodents is worrying the researchers who study immunity. Indeed, generation of controversial data could result from the great difference in the composition of the DM of rodents coming from one breeding unit or another, and which housing conditions are very diverse. In the future, a standardisation of the DM colonising experimental animals has to be defined.

Organisms of questionable significance

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What is clean? The FELASA Recommendations for health monitoring of rodents state "it is not a requirement of these recommendations that animals tested are free from all of the microorganisms tested." However, facility managers and investigators, concerned about maintaining the health status of their facilities or the possible introduction of variables to their research projects, generally require health monitoring reports for animals they receive to be negative for all the agents listed. Some of these organisms are of questionable significance, yet may lead to the termination of the colony and to a delay of important investigations, even though the organisms have no known impact on research use. We know much less about the thousands of species that make up the intrinsic microbiota than we know about the few microbes that cause disease. Animals, including humans, have evolved with diverse groups of microorganisms that are required for normal health and development. These organisms are friends and foes. Some, for example, are important for stimulation of the immune system and it is safe to say that all aspects of animal host biology can be impacted. Some examples of such organisms are: some *Pasteurellaceae* [*Pasteurella sp*] in rodents, *Bordetella bronchiseptica* in rodents, rabbits and guinea pigs, *Corynebacterium urealyticum* in nude mice, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in immunocompetent animals, some *Helicobacter spp* in immunocompetent animals, *Streptococcus pneumoniae* in the mouse, and *Actinobacillus, Proteus*, *Klebsiella sp.*...Organisms, for which pathogenicity has been attributed in the older literature, may no longer be pathogenic or capable of causing effects on research in animals that are of otherwise high health status. Experiences with *Bordetella bronchiseptica* in guinea pigs have shown that, whilst the organism may be cultured and antibodies detected by serology, clinical or experimental changes are absent. The same may be said of some *Pasteurella pneumotropica* infections in rodents and some protozoan infestations, such as *Entamoeba spp*, *Chilomastix spp.*, and other nonpathogenic flagellates. *Helicobacter spp* have presented universal challenges to researchers and to facility managers who are concerned regarding the conflicting reports of pathogenicity and non-pathogenicity. Should users be concerned regarding the presence of those *Helicobacter spp* for which no significance has been demonstrated and which may be commensal? Housing systems, and procedures related to the production of immunocompetent and immunodeficient animals are clearly of importance in the maintenance of appropriate microbiological status. However research facilities may not have the capability to maintain animals free from the subject organisms and the higher production costs may not be justified. It is suggested that future reviews of FELASA recommendations should provide some additional guidance on such potentially obsolete organisms.

Gnotobiology applied to the production and experimental use of human disease models, practical applications and selected examples

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The use of transgenic technology and other emerging models became a key component to "rational drug discovery approach" and biomedical research. Any institution or company involved in creation, development and production projects should address all relevant issues: genetics, microbial status, diet and environment control....

This presentation is focusing on health definition, management and control or in other words, gnotobiology applied to rodent models of human diseases.

Some of these rodent models are either immunocompromised or can be significantly fragilised and more sensitive to various microbial agents.

In other cases, the environmental microbism is a potential interfering factor with the experimental application(s).

As a consequence, before deciding about the health definition and programme suitable to an animal model it is critical to review both its specific sensitivity and the experimental requirements.

A series of case studies will illustrate situations of positive and negative experimental interaction between the microbial status and the phenotype or the experimental outcome.

Some key steps of a global programme for applied gnotobiology management will be reviewed.

This paper and its conclusion will address the quality level and control of a health standard depending on the type of model, its immunocompetency and its experimental use.

Biological test systems need to be clearly defined

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Although the use of laboratory animals for research is highly regulated, animals specifically used in testing (biological test systems) are under particular scrutiny. This is not only the case for their animal welfare and husbandry, but because they will generate test results in support of the development of drugs and devices used in the fields of e.g. human and veterinary medicine. The companies involved in the processes of testing and reviewing of data have the double onus to certify the validity of the data generated and to make scientific evaluations of the test findings in reference to future applications. Live animals are a necessary yet complex test system. Any environmental or man made changes to these test systems could have repercussions downstream, and hence confound the interpretation of the physiological parameters that are typically assessed. Therefore, any modification needs to be fully understood with regards to quality and magnitude of its potential impact on the test system. Biomedical research projects using animals should be conducted under standardized, rigorous conditions in order to generate reliable data that can be scientifically and soundly interpreted. To achieve this goal, we need to start with a genetically stable, highly defined test system when possible, and carefully monitor each environmental and technical factor throughout the experimental process.

Communication Management in Biomedical Research

Pharmaceutical industry and animal welfare organisations work towards the same objective

Gabriele Küsters, Aventis, and Magda Chlebus Research and Animal Welfare Group EFPIA

The attitude of politicians and society towards animal research is getting more and more complex and ambiguous due to constant antagonism between environmental and consumer policies and research and industrial policies. This trend is visible at both national and European level. However, the opinion polls seem to indicate a support for animal research for medical purposes and one should not forget that human and animal health is the ultimate goal of pharmaceutical research.

Moderate animal welfare organisations on the one hand, and veterinarians/scientists working in animal laboratories on the other have much in common - both have a mutual goal - humane care for experimental animals:

-to improve living and housing conditions

-to minimise the number of animals used -to ensure that, where possible, alternatives to animals are developed and used.

The only differences between the respective approaches are that

-Pharmaceutical industry has to take into account legislative reasons (we are obliged by law to do animal research) and economic reasons (as an industry we have to be profitable) in addition to ethical values,

-Pharmaceutical industry not only bears a moral responsibility for experimental animals, but also for human and animal health (it is our task to develop new and better medicines).

These approaches are not mutually exclusive, but complementary. Responsible welfare organisations can help by focussing on issues that scientists, governments and authorities could further investigate and welfare organisations can and should work in partnership.

The misunderstanding surrounding the industry role results from rather reactive than proactive communication on biomedical research in general, and animal research in particular, thereby allowing opponents have the field to themselves. Industry's failure to engage in dialogue due to the pressure of anti-animal experimentation activism and terrorism directed at companies and individuals in the early days has left it playing catch-up with the activists ever since.

This is changing slowly but surely by means of

-dialogue with all stakeholders on research into treatments requiring animal research and application of the 3Rs

-involvement in responsible decision-making

-promotion of highest standards among industry laboratories and animal housing (e.g. AAALAC accreditation, FELASA training, etc.)

cHow do we do it? Through:

-EFPIA Research and Animal Welfare Group activities

-Responsible involvement in the TEWG (EU Technical Expert Working Group)

-Projects on environmental enrichment

-Publications

Internal communication in a global Pharmaceutical company

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Summary

Pharmaceutical companies have assumed global proportions with numbers of employees reaching well above 50,000. These people carry out a wide range of tasks, the majority of which are far removed from research and development and from the use of animals in particular. The employees at a global company represent people from all walks of life with interests and biases seen in any cross section of society. Conversations with non-scientist employees often reveal that people who do not work closely with R&D often are ignorant about the fact that animals are used, and even more ignorant about the necessity for their use. Many express surprise and in a few cases are negative to their use. The responsible use of animals use of animals is central to successful R&D. It is essential that companies inform their employees about their use and in so doing dispel many of the misgivings they may have. A successful communications program will provide all categories of personnel with information they can use at their workplace and at home. Company employees are important ambassadors and, if provided with appropriate information, will use it actively in supporting their company's activities, including the use of animals. Even more important is that employees who previous to being informed may have been negative or even hostile to the use of animals may change their opinion and assume a neutral to positive attitude. This presentation will describe programs given at several large global pharmaceutical companies.

Large pharmaceutical companies are multinational and spread worldwide. These companies employ many tens of thousands of employees with administrative offices, research sites, and manufacturing plants located in almost every country found as a member of the United Nations. Employee roles range across a wide spectrum of activities and skills including lawyers, engineers, chemists, truck drivers, biologists, veterinarians and medical doctors to mention but a few. In many cases employee groups work within a compartmentalised structure. The sheer size of the organisation is such that many of them are not aware of the nature of work roles outside of their own sphere of activity. Attention in a large pharmaceutical company is focussed on the product – drugs brought to market for use in a patient population. The route to final product and the logistics surrounding drug discovery and development is long and at time tortuous. Many disciplines and skills are involved involving both scientists and non-scientists. Research and drug development lie at the core of all successful large drug companies. Despite this, conversations with non-scientist employees often reveal that people who do not work closely with R&D often are ignorant about the multidisciplinary nature of the work. Many employees are not aware of the fact that animals are used in R&D. To those of us who work closely with animals this may come as a bit of a surprise. Conversations with our non-scientist colleagues (and for that matter with scientists who do not work with animals but are involved in R&D) uncover attitudes that range from ignorance and indifference or surprise, to negative views. This should not be totally unexpected. The employees at a global company will come from many walks of life and have many backgrounds. They will reflect the attitudes seen in society at large and as such will express a similar range of opinions. The responsible use of animals is central to successful R&D. It is essential that employees understand this and feel comfortable with the knowledge.

The use of animals engages people and will often lead to lively and at times heated discussion. Industry has powerful arguments in support of the responsible use of animals in research and development:

- Research carried out using animals benefits human and animals alike.
- Industry has the highest standards of animal care & welfare
- Animals are used following rigorous ethical assessment and justification for their use
- Industry assigns the care of the animals to highly trained professionals (veterinarians and caretakers)

With this in mind employees at pharmaceutical companies could, if properly informed and trained, act as ambassadors for the use of animals in research. People who may have a negative attitude could have their misgivings assuaged and at best be given a positive view. Society in general demands more openness and a "right to know". Our employees as members of society have the same "right" to know about the use of animals in industry. This also extends to employees who work with animals, and who would in many cases benefit from more in-depth knowledge. Providing information to employees outside the R&D community will relieve their concerns and give confidence that the use of animals in industry is not trivial. Experience shows that many employees who work with animals are reticent to tell even their family members that they do such work. Providing them with the right information will go a long way in alleviating this.

Internal communication programs should be directed at several different audiences.

- Staff who have their daily work with laboratory animals gain through:
  - access to more information on the work being done
  - access to Q&A's designed to facilitate communication with their peers
  - access to communication strategies for family members
- Scientists who use animals in their R&D activities benefit by:
- understanding the impact of animal use on society at large and by implication
- understanding the impact on the wider company employee community
- honing their communication skills and popularise their

work thereby allowing laypeople to understand what they are doing, thereby facilitating the message that their work benefits society at large
- Non-scientist employees gain confidence in the care and use of animals by:
- insight into animal care and use programs – that there is nothing to hide
- understanding the key role animals play in R&D
- understanding that the use of animals is not trivial
- understanding that animals are cared for by dedicated and professional people

Company training programs

Several large multinational pharmaceutical companies have introduced global employee communications programs designed to fulfil these aims. Three programs are described:

1. Pfizer

Pfizer aims to inform and increase colleagues' awareness and understanding of the issues surrounding the use and welfare of animals in medical research by:
- Offering help, support and guidance to a section of colleagues who may need to answer and manage frequently asked questions
- Keeping colleagues appropriately informed/updated about Company policies
- Encouraging best care and welfare practices
- Providing a general awareness around associated security issues

Pfizer has developed a set of printed materials (which are revised and updated)
- Policies/positions
- Informative brochures/leaflets (including information for new starters)
- Frequently asked questions documents
- Animal welfare posters

Face-to-face internal communication initiatives available to staff include:
- 60 Minute lectures/presentations – with interactive Q&A sessions
- Communication/updates on associated initiatives across business divisions
- Colleague training for school speaker programmes
- Visits and talks given by representatives of external groups,

Pfizer also has an intranet site covering a range of issueson animal care and welfare.

2.Aventis Pharma

Aventis Pharma has established a global modular information campaign that is offered to associates across a wide range of functions. The program is a collaboration between members of the internal laboratory animal expert group and external organisations/associations in the countries concerned. The program consists of a series of 60 minute presentation meetings are held at in-house venues and involve a\interactive Q&A sessions.

Four modules have been organised

- Level One: Plenum lecture on the use of animals at the company
- Aim: staff should understand what animal welfare and animal rights are about, how media public opinion works and what impact the critics may have on legislation and the company's work
- Level two: Workshop
- Aim: smaller groups of staff leant more in-depth detail, people suited for further training (media or lecturing) are identified
- Level three: One-day seminar
- Aim: staff should be able to answer questions on animal welfare, issues to family members and friends, and to handle critical argument. Understand the principles behind the rationale for the use of animals in science and to popularise these
- Level four: 2.5 days of media training (external venue)
- Aim: staff should be able to meet critics and journalists in front of a TV camera

Aventis has organised level one meetings for Non-R&D administration centres. An interactive CD-ROM and Q&A card game is available for French speaking employees and will be translated into the two other company languages. The program is supported by tours through the animal facilities

The program has elicited very positive responses from all categories of employees. In keeping with a general impression may employees voice surprise on hearing that the company uses animals in R&D. There is a unison request for more insight and information. Employees invariably state that access to information allows them to speak more openly and frankly when talking about their workplace at home or with friends. Employees who undergo TV and media training say this is positive and builds confidence both at a personal and workplace level. A key take-home message is that the program needs to be targeted according to country and culture in order to be successful. A detailed Intranet site that offers additional information at several levels supports the program.

3. GlaxoSmithKline.

GlaxoSmithKline has had long experience in organising internal and external communication and training sessions. A person with teaching skill is employed by the Laboratory Animal Department and is responsible for planning and coordination associate training. They have included a mandatory 30 minute session on the use of animals in R&D as part of the Global new employee induction program.

Tours are arranged through the animal facilities for internal and external participants. Animal facility staff have been trained to interact with associates and to feel at ease with questions when asked. Techniques are given for interaction with friends and family. Brochures and printed materials are available as well as school sets that can be used by associates when visiting schools and community centres. Animal welfare posters are available, media training is given and key personnel are trained to interact with journalists. GlaxoSmithKline has an intranet site that focuses on a wide range of issues including Q&A's and discussion topics as well as a description of the use of animals in R&D.

The Internet

The Internet is a significant source of information to employees and to the public at large. The sites of several companies have been surveyed and benchmarked – sites were surveyed for mention of animal experiments, reduction and alternative methods, number of animals used specified and the presence of images. The list in table 1 is not complete but gives an indication of the level of information that can be gained from such sites:

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Table 1:Overview of a selection of Pharmaceutical Company Internet sites describing the use of animals in research & development

Company	Animal experiments mentioned	Reductions & Alternatives mentioned	Number of animals specified	Images of animals
Abbot	no	no	no	no
Altana	no	no	no	no
Amgen	yes	no	no	no
AstraZeneca	yes	yes	yes	no
Aventis	yes	yes	no	yes (1 photo)
Bayer	no	no	no	no
Böhringer	yes	yes	no	no
Bristol-Myers	yes	yes	no	no
Eli Lilly	yes	yes	no	no
Glaxo SmithKline	yes	yes	no (only%)	no
Merck	yes	yes	no	no
Novartis	yes	yes	no (only %)	no
NovoNordisk	yes	yes	no	no
Pfizer	yes	yes	no	no
Procter & Gamble	yes	yes	no	no
Roche	yes	yes	no	no
Sanofi-Synthelabo	yes	yes	no	no
Schering-Plough	yes	no	no	no
Takeda	no	no	no	no
Wyeth	yes	no	no	no

Courtesy of Dr. Tilmann Kiessling Corporate Communications Aventis Pharma

Internal communication in a public institution

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During the past 50 years, current relationships between human beings and other animals evolved under our growing understanding of animal cognition and suffering. Correlated with the development of new technologies (GMO animals, *in vivo* imaging, cell and gene therapy) this led to the establishment of overwhelming regulations and controls on animals, environment, and peoples preservation. Among its 13,500 employees, INSERM, The French National Institute for Health and Medical Resarch accounts for more than 3,500 researchers and technicians working on laboratory animals. Two hundred and fifty five animal facilities located all over the French territory are dedicated to the production, housing and all types of experimentation on most laboratory animal species. The Bureau de l'Éxpérimentation Animale (BEA) is in charge of the follow up of these peoples and facilities regulatory situation. It provides all necessary data and communications on regulations, ethical issue, technical and methodological development necessary to a good practice of laboratory animals experiments. In collaboration with the "bureau de l'évaluation des risques" it provides all the necessary clues on how to design specific facilities and procedure for specific activities i.e. biological hazards. In collaboration with the Département de la Communication, it develops documents for the external and internal communication on laboratory animals experiments. Some practical examples, will illustrate the specificities of the internal communication allowing the follow-up of laboratory animals experiment in this multicentric public institution.

How to inform the public on animal experimentation

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GIRCOR is an association under the 1901 Law, it gathers together all French public institutions and private companies which use animals intended for biological or biomedical research.

The purpose of GIRCOR is to make sure that the public's view on laboratory animals is as positive as possible.

This goal can be reached if the laboratories, when dealing with laboratory animals, apply principles that are as close as possible to people's expectations and let them know about it.

This implies various actions which should be taken such as opinion polls, development of ethical committees, contacts with opinion intermediaries (press, associations for animal protection), distribution of leaflets, and training of researchers on communication with the public.

The most recent developments in this matter will be presented during this communication.

Communicating to European Institutions

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More and more national legislation is influenced, shaped or entirely determined by EU legislation. As a result, many organisations that have a legitimate interest in national legislation have realised that they not only need to be aware of what is happening in Europe but also need to be able to influence it.

So, which European institutions should you be communicating with? This is fairly easy to work out. If you are concerned with primary European legislation such as Directives and Conventions, you need to be communicating with the various parts of the EU and Council of Europe. Within the EU, there are three crucial parts: the European Commission, the Council of Ministers and COREPER and the European Parliament. Most of the comments in this paper are directed at communicating with these institutions. It is somewhat easier to manage communications with the European Parliament than with the other institutions. Normally, one is dealing with individual MEPs, who are happy to discuss issues with a wide range of organisations and individuals. However, there are a number of principles that apply when communicating with other European institutions.

The first stumbling block that many organisations discover is that it is very difficult – often impossible – to communicate effectively with European institutions if you are a national organisation. To be effective, you need a European platform. In some cases, there may be a European organisation with sufficient interest in your area that you can use it as a platform. It may simply be necessary to approach the existing organisation and tell them about the issue that you are concerned about and they will agree to adopt it as one of their actives issues for EU lobbying. There can be both advantages and disadvantages to this approach. An established European organisation is likely to already have the knowledge, skills and standing to communicate effectively within the EU. On the other hand, you may have to give them control of the communications on this issue, and accept that it has to fit in with their other priorities and policies.

For these reasons, it is often decided to set up a new platform for communicating within Europe. Sometimes, a group of analogous national organisations in different EU countries will come together to form a federation (e.g. FELASA) or, if there are insufficient national organisations, it may be necessary to set up an independent European organisation with analogous objectives (e.g. EBRA). The end result is the same: a new European organisation.

The next step is to ensure that your new European organisation has credibility and standing. There are many tried and tested methods of achieving this. You should ensure that the launch of your new organisation is given the right publicity. This means publicity that reaches your key audiences: your own stakeholder group (eg laboratory animal scientists, animal researchers, etc) and the relevant officials in European institutions. At the simplest level, it is always worth writing to all the relevant officials, not only in the EU and Council of Europe, but also in the relevant European trade associations, regulatory agencies and other interest groups, to introduce your new organisation.

If you are fortunate, it may be possible for your new association to get EU funding to carry out a specific function that will raise its profile. Such things can include running a conference, carrying out a survey, producing a report and so on. How do you go about this? Such opportunities are not often advertised and sometimes only exist as someone's idea, so you have to spend time networking with your new colleagues in the European institutions. Identify who they are, visit them, discuss your concerns and their concerns, look for how they overlap and do not be shy about suggesting things that you could do. For many European officials, a significant part of their job is giving grants for various purposes. Advising someone about how they can apply for funding is an entirely normal part of their work.

One of the most important aspects of communicating with EU institutions is to identify the key people – often the key person – within a particular institution who is responsible for the issue that concerns you. You need to know these people. You need to make sure they know you. Talk to them. When you are at meetings together, get to know them. However, tread carefully – the very last thing you want to do is to appear pushy or annoying to them. Just like you, they are busy people who will not like having their time wasted. A good working relationship with the key officials, based on mutual respect, is a huge advantage in communicating with the European institutions.

Much of the most important communication takes place in specific meetings or groupings. The membership of these is normally composed of representatives of the key stakeholder groups. It is crucial that your organisation is such a group and always has a place at these meetings. At the Council of Europe, the Multi-Lateral Consultation meetings have been revising the caging, husbandry and housing standards for laboratory animals across Europe for the last few years. At the EU level, the impending revision of Directive 86/609 was started with a Technical Expert Working Group that produced a series of reports that will strongly influence the content and shape of the new, revised directive. Sometimes, it is worth setting up your own meetings on the issue which you are concerned about and inviting other key stakeholders, MEPs and European officials to attend and speak.

Finally, it is important not to be too narrow in your communications within Europe. The formal responsibility for the regulation of animal research may rest with DG Environment, but there are many other Directorates-General (and many other parts of the European Parliament, the Council of Ministers and COREPER) who have a legitimate interest and influence. Animal experimentation is relevant to health, to industry and to research. The relevant sections of these different organisations have to be consulted. There are European trade associations and stakeholder groups that represent these interests who should be involved. For the most effective communication with European institutions, you have to be as inclusive as possible.

Crisis communication training in laboratory animal care issues, advantages of a simulation exercise

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Public or private research laboratories sometimes face violent attacks from animal activists. All are supposed to have access to tailored procedures to prevent and manage such crisis situations (alert, security, emergewncy communications, media management ...)

These procedures must be tested on a routine basis through a practical simulation exercise to remain really effective at any time.

An effective crisis exercisse requires writing up a realistic scenario taking into account both the specifics of the laboratory or company concerned and the media and cultural background. The crisis team must have been identified in advance and trained in crisis management.

A realistic exercise will confront them with emergency decisions ans well as a huge number of phone calls coming from different stakeholders (public bodies, activists, media, consumers ....) . A half-day exercise will deliver profitable lessons to the entire team. Their capability to get organised, to become an effective working group and the relevance of the communicated messages are the areas where an exercise can highlight improvement areas. The demonstartion will be illustrated by a case study.

Issues management - proactive communications

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Animal welfare has been an important issue in Britain for well over 100 years, whereas the animal rights (AR) movement originated in the 1960s with the campaigning and associated activism beginning in the 70s. Since then there have been peaks and troughs of extremist activity targeted at many organisations invoved in biomedical research, including university and government laboratories, pharmaceutical companies, contract research organisations and laboratory animal breeders.

In the last few years we have been experiencing one of these peaks of activism and this has affected many organisations, including Huntingdon Life Sciences (HLS). Activist tactics have developed and. as a result, many stakeholders of these organisations have been targeted, so becoming secondary targets, even though they are not directly involved in animal research themselves.

At HLS we believed that openness was essential if we were to play a leading role in improving the UK public's understanding of animal research and of how that research can benefit society. This openness helped to inform many visitors including politicians and the media, and allowed them to appreciate that AR campaign literature is generally misinformation and sensationalist propaganda. Many other organisations also successfully communicate with the public on this issue and together we realised that a broad alliance of research organisations could add further credibility to the case for animals research. The alliance, the Coalition for Medical Progress (CMP) was launched in 2003 and includes representatives from academia, charities, unions, government and industry. The CMP has already made a number of additional, positive contributioons to the public communication on the use of animals in biomedical research.