ANIMAL MODELS IN ANALGESIC DRUG DEVELOPMENT

Parisa Gazerani, Pharm D, PhD, SMI®, Department of Health Science and Technology
Faculty of Medicine, Aalborg University Frederik Bajers Vej 7A (A2-208) Phone: 9940 2412 Email: gazerani@hst.aau.dk
AGENDA

• Explain the ethical aspects of animal testing for the safety and effectiveness of new drugs

• Have knowledge of biomarkers in preclinical stages and skills to translation it to human trials

• Describe and discuss relevant models, systems, and tools in preclinical studies in efficiency, safety and interactions

• The ability to account for national and international authorities in relation to drug approval and development

• Analyze risks and challenges in preclinical development and come up with solution ideas

Pain a and analgesia are in focus
Case II - group work to practice

- You are working as a consultant for BBB Pharma Company specifically focused on developing analgesic compounds for targeting pain in humans.

- Two weeks from now you have a meeting with the company pre-clinical phase head officer and need to present your animal model to test the efficacy of the new pain killer called AAU007!

- Discuss in your group and make a plan on how would you pick up a model and design tests for efficacy of AAU007.

Questions:

- Which points you will consider the most important ones in selecting a model?

- Do you see some challenges? Where?

- What strategy would you consider to overcome those challenges?
PRECLINICAL EVALUATION

1. **What** is preclinical evaluation?
2. **Why** we need to do it?
3. **When** we do it?
4. **How** we do it?
**WHAT IS PRECLINICAL EVALUATION?**

- When developing a potential new pharmaceutical compound *(or a medical device)*, the primary objectives are to demonstrate that under the conditions of therapy, the potential new drug is effective and safe.

- Thus, preclinical evaluation is to test the **safety** and **efficacy** in appropriate animal or *in vitro* models (New technologies are nowadays available: Omics: toxicogenomics, toxicoproteomics).
WE NEED TO DO PRECLINICAL EVALUATIONS,

WHY?

• Preclinical studies were relatively superficial until several disasters had occurred! (thalidomide catastrophe in the 1960s).

• To avoid more disasters! today there are national and international regulations that require manufacturers to provide information from a detailed package of preclinical studies.
WE DO PRECLINICAL EVALUATIONS, WHEN?

- Before first human use!
Regulatory agencies around the world have issued guidelines, which lay down their expectations of a thorough testing and assessment program (ICH guideline).

1. Safety pharmacology – indication of adverse pharmacologically mediated actions on central nervous, cardiovascular and respiratory systems
2. Pharmacokinetics – preliminary studies on absorption, distribution, metabolism and excretion
3. Acute toxicity information – two species either assessed directly or by inference from data of the highest tolerable doses in range finding studies. Usually an evaluation of the maximum repeatable dose (MRD) and possibly local irritancy
4. Repeat-dose toxicity – rodent and non-rodent species are required. The duration of the test depends on the duration of clinical exposure but many companies conduct two 14-day studies before going into humans. Studies should be performed using the proposed clinical route and at least one species should be a pharmacologically responsive species that expresses the target pathway of the investigational drug
5. Reproductive toxicology – usually embryo/fetal development studies in two species are required in Europe and Japan if women of child-bearing potential are included. Not required in the USA for some early trials
6. Mutagenicity – tests for mutagenicity and chromosome damage
PRECLINICAL EVALUATIONS: GUIDELINES

The European Medicines Agency’s scientific guidelines on the non-clinical testing of medicines help applicants prepare marketing authorisation applications. Guidelines reflect a harmonised approach of the EU Member States and the Agency on how to interpret and apply the requirements for the demonstration of quality, safety and efficacy set out in the Community directives.

The Agency strongly encourages applicants and marketing authorisation holders to follow these guidelines. Applicants need to justify deviations from guidelines fully in their applications at the time of submission. Before that, they should seek scientific advice, to discuss any proposed deviations during medicine development.

Non-clinical guidelines are provided for:
- Environmental risk assessment
- Non-clinical development
- Pharmacokinetics and toxicokinetics
- Pharmacology and safety pharmacology
- Toxicology

How helpful is this page?

Average rating: 

Based on 11 ratings

Add your rating:

See all ratings

Non-clinical: toxicology

The European Medicines Agency's scientific guidelines on toxicology help medicine developers prepare marketing authorisation applications for human medicines.

If you have comments on a document which is open for consultation, use the form for submission of comments on scientific guidelines.

For a complete list of scientific guidelines currently open for consultation, see Public consultations.

- Single and repeat-dose toxicity
- Genotoxicity
- Carcinogenicity
- Reproductive/developmental and juvenile toxicity
- Local tolerance
- Other toxicity
- Specific types of products

Single and repeat-dose toxicity

- Questions and answers on the withdrawal of the 'Note for guidance on single dose toxicity'
- Repeated dose toxicity
- ICH S4 Duration of chronic toxicity testing in animals (rodent and non-rodent toxicity testing)

Genotoxicity

- ICH S2 (R1) Genotoxicity testing and data interpretation for pharmaceuticals intended for human use
- ICH M7 Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk
- Assessment of the genotoxic potential of antisense oligodeoxynucleotides
- Genotoxic and carcinogenic potential of phenolphthalein

Carcinogenicity

Good laboratory practice compliance

This content applies to human and veterinary medicines.

The principles of Good Laboratory Practice (GLP) define a set of rules and criteria for a quality system concerned with the organisational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, reported and archived.

Exhaustive information about GLP can be found on the websites of the [OECD](http://www.oecd.org) and the European Commission. The GLP Directives are applicable: Directive 2004/9/EC and Directive 2004/10/EC.

Questions and answers concerning the interpretation of the two GLP Directives can be found in Questions and answers concerning the Implementation of Directives 2004/9/EC and 2004/10/EC on Good Laboratory Practice. The questions were discussed by Commission services and representatives from the Member States' GLP-monitoring authorities and the answers were approved by the EU GLP Working Group. The document attempts to provide guidance to monitoring authorities, regulatory authorities and test facilities. The answers represent the opinion of the EU GLP Working Group.

GLP Inspections

The Procedure for co-ordination of GLP inspections describes the co-ordination of GLP inspections of the non-clinical safety, toxicological and pharmacological studies proposed in human and veterinary applications for marketing authorisations and various post-authorisation applications submitted to the European Medicines Agency through the centralised procedure. It is effective as of 27 September 2012.

The European Medicines Agency has a co-ordinating role for these inspections, whilst the responsibility for carrying them out rests with the Competent Authority under whose responsibility the test facility falls.

Ad hoc GLP Inspectors Working Group

The ad hoc GLP Inspectors Working Group focuses on harmonisation and coordination of GLP-related activities at Community level. EMA's Clinical and Non-clinical Compliance Service chairs and provides secretarial support to the ad hoc GLP Inspectors Working Group.
Guidances (Drugs)

About FDA Guidances
Guidance documents represent the Agency’s current thinking on a particular subject. They do not create or confer any rights for or on any person and do not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both. For information on a specific guidance document, please contact the originating office. Another method of obtaining guidance documents is through the Division of Drug Information.

Search all FDA Guidances

Good Guidance Practices and Agenda
- FDA’s Good Guidance Practices regulation (PDF - 162KB) September 2000
- Guidance Agenda: Guidance CDER is Planning to Develop During Calendar Year 2017 (PDF - 45KB) (updated 04/19/2017)
- How to comment on Guidance Documents

Newly Added and Withdrawn Guidances
- Newly Added Guidance Documents

Quick links
- Guidance Agenda 2017 (PDF - 45KB)
- Newly Added Guidance Documents
- Product-Specific Guidelines for Generic Drug Development
- Spanish Language Guidelines

Contact FDA
- For more assistance, explore ways to contact the FDA

## International Council for Harmonisation - Safety

Below is a sortable list of the International Council on Harmonisation - Safety Guidance Documents. You can sort alphabetically by: Category/Subject Area; Guidance Title; Guidance Type (Draft or Final) and also sort by the Date the Guidance was issued.

The International Conference on Harmonisation has changed its name to International Council for Harmonisation

<table>
<thead>
<tr>
<th>Category</th>
<th>Title</th>
<th>Type</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>International Council on Harmonisation - Safety</td>
<td>S1A The Need for Long-term Rodent Carcinogenicity Studies of Pharmaceuticals (PDF - 100KB)</td>
<td>Final Guidance</td>
<td>03/01/96</td>
</tr>
<tr>
<td>International Council on Harmonisation - Safety</td>
<td>S1B Testing for Carcinogenicity of Pharmaceuticals (PDF - 145KB)</td>
<td>Final Guidance</td>
<td>02/28/95</td>
</tr>
<tr>
<td>International Council on Harmonisation - Safety</td>
<td>S1C(R2) Dose Selection for Carcinogenicity Studies of Pharmaceuticals (PDF - 185KB)</td>
<td>Final Guidance</td>
<td>05/17/93</td>
</tr>
<tr>
<td>International Council on Harmonisation - Safety</td>
<td>S2A Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (PDF - 123KB)</td>
<td>Final Guidance</td>
<td>04/01/96</td>
</tr>
</tbody>
</table>

---

Below is a sortable table of Pharmacology/Toxicology guidances.

<table>
<thead>
<tr>
<th>Category</th>
<th>Title</th>
<th>Type</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmacology/Toxicology</td>
<td>Carcinogenicity Study Protocol Submissions (PDF - 29KB)</td>
<td>Final Guidance</td>
<td>05/22/02</td>
</tr>
<tr>
<td>Clinical/Medical; Investigational New Drug Applications; Pharmacology/Toxicology</td>
<td>Content and Format of Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs, Including Well-Characterized, Therapeutic, Biotechnology-derived Products (PDF - 42KB)</td>
<td>Final Guidance</td>
<td>11/01/95</td>
</tr>
<tr>
<td>Clinical/Medical; Investigational New Drug Applications; Pharmacology/Toxicology</td>
<td>Content and Format of INDs for Phase 1 Studies of Drugs, Including Well-Characterized, Therapeutic, Biotechnology-Derived Products, Questions and Answers (PDF - 14KB)</td>
<td>Final Guidance</td>
<td>10/01/00</td>
</tr>
<tr>
<td>Pharmacology/Toxicology</td>
<td>Developing Medical Imaging Drug and Biological Products Part 1 Conducting Safety Assessments (PDF - 271KB)</td>
<td>Final Guidance</td>
<td>06/17/04</td>
</tr>
<tr>
<td>Pharmacology/Toxicology</td>
<td>Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers (PDF - 702KB)</td>
<td>Final Guidance</td>
<td>07/26/05</td>
</tr>
<tr>
<td>Investigational New Drug Applications</td>
<td>Exploratory IND Studies (PDF - 220KB)</td>
<td>Final Guidance</td>
<td>01/12/06</td>
</tr>
<tr>
<td>Pharmacology/Toxicology</td>
<td>Format and Content of the Nonclinical Pharmacology/Toxicology Section of an Application* (PDF - 1,3MB)</td>
<td>Final Guidance</td>
<td>02/01/07</td>
</tr>
</tbody>
</table>
Electronic Code of Federal Regulations

**e-CFR data is current as of July 20, 2017**

<table>
<thead>
<tr>
<th>Title</th>
<th>Volume</th>
<th>Chapter</th>
<th>Browse Parts</th>
<th>Regulatory Entity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title 21</td>
<td>1</td>
<td>1-99</td>
<td>1-99</td>
<td>FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN SERVICES</td>
</tr>
<tr>
<td>Food and Drugs</td>
<td>2</td>
<td>100-169</td>
<td>100-169</td>
<td>DRUG ENFORCEMENT ADMINISTRATION, DEPARTMENT OF JUSTICE</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>170-199</td>
<td>170-199</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>200-299</td>
<td>200-299</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>300-499</td>
<td>300-499</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>500-599</td>
<td>500-599</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>600-799</td>
<td>600-799</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>800-1299</td>
<td>800-1299</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>1300-1399</td>
<td>1300-1399</td>
<td>OFFICE OF NATIONAL DRUG CONTROL POLICY</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>1400-1499</td>
<td>1400-1499</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Need assistance?

https://www.ecfr.gov/cgi-bin/text-idx?SID=3ee286332416f26a91d9e6d786a604ab&mc=true&tpl=/ecfrbrowse/Title21/21tab_02.tpl
Summary: Preclinical safety assessments

- **What?** Tests in preclinical evaluation of new medicinal products
- **Why?** Preclinical safety and toxicity matters!
- **When?** Preclinical stage of drug or medical device development
- **How?** Safety pharmacology tests, toxicology tests (see examples of tests, dose, teratogenicity, cytotoxicity, ...) in animals (see type of animal for different tests) based on ICH
- **Biomarker?** TD50, histopathology, electrocardiography, ...
STOP

TAKE A BREAK
ETHICAL RULES

ANIMAL EXPERIMENTATION
Controversial topic of animal experimentation...

- Animal experiments can benefit humans
- Animal experiments harm the animals
EXPERIMENTATIONS ON ANIMALS

WHY?

• Retrospective studies show that the detection of clinically relevant toxicities by preclinical animal studies is high and useful, although it is by no means 100%.

• Minimization of animal use is an established goal of the ICH guideline process. It has been estimated that the number of animals used in toxicology for registration of a ‘standard’ compound can be reduced by 50%.

• In the future it is possible that the need for animal studies will be further minimized by:
  • the use of low and ultra-low dose studies in humans
  • use of the ‘omic’ technologies will also increase over time
Why Animals Are Needed in Research

https://www.youtube.com/watch?feature=player_embedded&v=iA_FfVuTfoM

4:30 min
DO WE STILL NEED ANIMAL MODELS?

WHY?

- Despite new and refined alternative methods, animal experiments will remain essential in the foreseeable future for biomedical research.

It may be acceptable to use animals for research, if:

- There must not be another way to achieve the same results which is less harmful to the animals
- The research must be of importance to the aim of finding new ways of preventing, curing and alleviating serious human diseases

key issues: Basel declaration, The three R’s (Reduce/Refine/Replace), ...

How to address the ethical issues?
ANIMAL EXPERIMENTATION - ETHICS

Contents:
Introduction
What are our Duties to Animals?
Does Animal Species Matter?
How can Benefits be Maximized?
How can Harm be Minimized?
How to Maintain Standards?

How to apply animal ethics, legislations and rules for experimentations on animals?
Like the Helsinki Declaration, which forever altered the ethical landscape of human clinical research, the aim of the Basel Declaration is to bring the scientific community together to further advance the implementation of ethical principles such as the 3Rs whenever animals are being used and to call for more trust, transparency and communication on the sensitive topic of animals in research.

The Basel Declaration Society, founded on October 5th. 2011, strive to promote the Basel Declaration.
Fundamental principles

We, the undersigned, shall:

1. Respect and protect the animals entrusted to us and not inflict unnecessary pain, suffering, or harm to them by adhering to highest standards of experimental design and animal care.

2. Consider carefully whether research involving animals addresses questions of importance that cannot be answered using alternative methods.

3. Strive to minimize the number of animals used for research and use the most suitable species to achieve the intended gain of knowledge.

4. Encourage collaboration to avoid repetition of animal experiments.

5. Implement the highest standards for protection of environment and public health.

6. Balance the interests of patients and society with our responsibility towards the animals when developing genetically modified animals.

7. Implement the highest standards of education and training for all persons who work with animals and monitor their compliance with standards on a regular basis.

8. Adequately recognize the important engagement of scientists in their efforts to promote the public understanding of science.

9. Promote the dialogue concerning animal welfare in research by transparent and fact-based communications to the public.

10. Provide advice based on scientific knowledge and expertise to political decision makers and government authorities on issues of research involving animals and their welfare.
II. 3Rs

Definitions

Methods which avoid or replace the use of animals
Replacement

Methods which minimise the number of animals used per experiment
Reduction

Methods which minimise suffering and improve animal welfare
Refinement

The ARRIVE guidelines
Animal Research: Reporting In Vivo Experiments

Carol Kilkenny1, William J Browne2, Innes C Cuthill3, Michael Emerson3 and Douglas G Altman3

1The National Centre for the Replacement, Refinement and Reduction of Animals in Research, London, UK; 2School of Veterinary Science, University of Bristol, Bristol, UK; 3School of Biological Sciences, University of Bristol, Bristol, UK; National Heart and Lung Institute, Imperial College London, UK; Centre for Statistics in Medicine, University of Oxford, Oxford, UK.

Animal research: Reporting in vivo experiments: The ARRIVE guidelines

Carol Kilkenny1, William Browne2, Innes C Cuthill3, Michael Emerson4 and Douglas G Altman3

1The National Centre for the Replacement, Refinement and Reduction of Animals in Research, London, UK; 2Department of Clinical Veterinary Science, University of Bristol, Bristol, UK; 3School of Biological Sciences, University of Bristol, Bristol, UK; National Heart and Lung Institute, Imperial College London, UK and 4Centre for Statistics in Medicine, University of Oxford, Oxford, UK.
Replacement

Replacement can be defined as methods, strategies or approaches which do not involve the use of live animals.

Replacement may be achieved through a number of tools or their combinations including:

- *in vitro* systems using tissues, whole cells or parts of cells
- systems based on biochemical approaches, i.e. using synthetic (macro)molecules as proxies of (reactive) toxicity targets. Such methods are referred to as "*in chimico*"
- computer-based models and approaches – often termed *in silico*
- use of 'omics' technologies (e.g. transcriptomics, proteomics and metabolomics)
- non-testing approaches such as 'read-across' technique

Reduction

The concept of reduction covers any approach that will result in fewer animals being used to achieve the same objective, including maximising the information obtained per animal, reducing the number of animals used in the original procedure and/or limiting or avoiding the subsequent use of additional animals.

Refinement

Today, the term refinement signifies the modification of any procedures or husbandry and care practices from the time the experimental animal is born until its death, so as to minimise the pain, suffering and distress experienced by the animal and enhance its wellbeing.

When an animal experiences pain, suffering or distress, there are often accompanying physiological changes which may increase the variability of scientific results. Refinement therefore is also likely to improve data quality and contribute to Reduction.

Photo credit: Novo Nordisk
<table>
<thead>
<tr>
<th>ITEM</th>
<th>RECOMMENDATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title</td>
<td>1 Provide an accurate and concise description of the content of the article as possible.</td>
</tr>
<tr>
<td>Abstract</td>
<td>2 Provide an accurate summary of the background, research objectives, including details of the species or strains of animals used, key methods, principal findings and conclusions of the study.</td>
</tr>
</tbody>
</table>

### INTRODUCTION

| Background | 3 a. Include sufficient scientific background, including relevant references, to previous work to understand the motivation and context for the study. Explain the experimental approach and rationale. b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study relevance to human biology. |
| Objectives | 4 Clearly describe the primary and any secondary objectives of the study, or specific hypothesis being tested. |

### METHODS

| Ethical statement | 6 Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal (Scientific Procedures) Act 1986) and national or institutional guidelines for the care and use of animals that cover the research. |
| Study design | 8 For each experiment, give brief details of the study design including: a. The number of experimental and control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment groups (randomisation procedure and when randomising results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be used to illustrate how complex study designs were carried out. |
| Experimental procedures | 7 For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anesthetic and analgesia used, including monitoring), surgical procedure, method of euthanasia. Provide details of any specialist equipment used, including specifications. b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific animal, route of administration, drug dose/used). |
| Experimental animals | 8 a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, International strain nomenclature, genetic modification status (e.g. knockout or transgenic), genotype, neurotransmitter, drug or cell name, previous procedures, etc. |

### Results

| Baseline data | 16 For each experimental group, report relevant characteristics and health status of animals (e.g. weight, morphological status, and drug or treat naïve) prior to treatment or testing (this information can often be tabulated). |
| Numbers analysed | 16 a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%). b. If any animals or data were not included in the analysis, explain why. |
| Outcome and estimation | 16 Report the results of each analysis carried out, within a measure of precision (e.g. standard error or confidence intervals). |
| Adverse events | 17 a. Give details of all important adverse events in each experimental group. b. Describe any modifications to the experimental protocol made to reduce adverse events. |

### Discussion

| Interpretation/scientific implications | 16 a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations, including any potential sources of bias, any limitations of the animal model, and the implications associated with the results. c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3R) of the use of animals in research. |
| Generalizability/translation | 16 a. a. Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology. |
| Funding | 20 List all funding sources (including grant number) and the name of the funder(s) in the study. |
LEGISLATION AND LICENSES RELATED TO ANIMAL EXPERIMENTATION - WHO?

Legislations:

• EU
• FELASA
• Danish Legislation

http://www.felasa.eu/
I. EU Legislations

Animals used for scientific purposes

Introduction

The protection and welfare of animals is an area covered by a wide range of EU legislation. This includes the protection of wildlife, zoo animals, farm animals, animals in transport and animals used for scientific purposes. Animal studies, whether for the development or production of new medicines, for physiological studies, for studying environmental effects or for the testing of chemicals or new food additives, has to be carried out in compliance with EU legislation.

Since 1986, the EU has had in place specific legislation covering the use of animals for scientific purposes. On 22 September 2010 the EU adopted Directive 2010/63/EU which updated and replaced the 1986 Directive 86/609/EEC on the protection of animals used for scientific purposes. The aim of the new Directive is to strengthen legislation, and improve the welfare of those animals still needed to be used, as well as to firmly anchor the principle of the Three Rs, to Replace, Reduce and Refine the use of animals, in EU legislation. Directive 2010/63/EU took full effect on 1 January 2013.

Latest updates

- **NEW** National statistical data on the use of animals as published by Member States has been updated with 2016 data from the UK
- **NEW** SCHEER has published its updated Opinion on "The need for non-human primates in biomedical research, production and testing of products and devices"
- **NEW** More popular Severity Assessment Workshops available - also in Italian and French – see details at our events page
- **NEW** Belgium has published an updated report on their Three Rs efforts
- **NEW** Several new interesting events have been added and the Italian PAREF representative updated
- **NEW** The Conference Report, presentations and posters are now available for the European Commission Scientific Conference: Non-Animal Approaches, The Way Forward

http://ec.europa.eu/environment/chemicals/lab_animals/home_en.htm
Legislation for the protection of animals used for scientific purposes

Horizontal legislation on the protection of animals used for scientific purposes

Directive 2010/63/EU

Directive 2010/63/EU revising Directive 86/609/EEC on the protection of animals used for scientific purposes was adopted on 22 September 2010. The Directive is firmly based on the principle of the Three Rs, to replace, reduce and refine the use of animals used for scientific purposes. The scope is now wider and includes foetuses of mammalian species in their last trimester of development and cephalopods, as well as animals used for the purposes of basic research, higher education and training. It lays down minimum standards for housing and care, regulates the use of animals through a systematic project evaluation requiring inter alia assessment of pain, suffering distress and lasting harm caused to the animals. It requires regular risk-based inspections and improves transparency through measures such as publication of non-technical project summaries and retrospective assessment. The development, validation and implementation of alternative methods is promoted through measures such as establishment of a Union reference laboratory for the validation of alternative methods supported by laboratories within Member States and requiring Member States to promote alternative methods at national level.

- Member State authorities for Directive 2010/63/EU

Consolidated Commission Implementing Decision 2012/702/EU as corrected by Decision 2014/11/EU

This Decision sets out a common format for submitting information on the use of animals for scientific purposes as referred to in paragraphs 1, 2, and 3 of Article 54 of Directive 2010/63/EU. The new system will allow the Commission to assess effectiveness of the implementation of the legislation and help ensure consistency in its application. The first data under the new statistical reporting format will be collected from 1 January 2014. The Seventh Statistical Report will follow

http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm
Animals used for scientific purposes

Upcoming events

- 20-24 August - 10th World Congress on Alternatives - Seattle, USA
- 6 September - LASA 3Rs/UFAW Section Meeting “Planning and implementing the 3Rs: Strengths, Weaknesses, Opportunities and Threats” - South of England
- 11-13 September - 55th Annual Meeting of the GV-Solas and 17th Advanced Training Course of the IGTP, including a Workshop on Severity Assessment Framework under Directive 2010/63/EU - Cologne, Germany
- 18-19 September - Fondazione Guido Bernardini Seminar "Cephalopod Molluscs in scientific research: relevance, challenges and opportunities" - Varese, Italy
- 25-26 October - 2nd RSPCA international meeting: Focus on severe suffering - Berlin, Germany
- 26-27 October - Harmonisation of the Care and Use of Wild and Domestic Mammals and Birds in Field Research - Oslo, Norway
- 7-8 November - The Danish 3R-symposium 2017 - Copenhagen, Denmark
- 28-30 November - LASA Annual Conference - Birmingham, England

Past events

- 6-7 December 2016 - Scientific Conference "Non-Animal Approaches - The Way Forward": Report and presentations - Brussels, Belgium

- 29-30 June - Fondazione Guido Bernardini Conference "Severity Classification of Animal Procedures" - Varese, Italy
- 22 June - LASA Care and Welfare Section "Not just skin deep – probing deeper with imaging – imaging applications in laboratory animal science" - Birmingham, England
II. FELASA

FELASA, the Federation of Laboratory Animal Science Associations, represents common interests in the furtherance of all aspects of laboratory animal science (LAS) in Europe and beyond. Membership is open to LAS associations of nations of Europe.
FELASA ACCREDITED COURSES

<table>
<thead>
<tr>
<th>FELASA ID</th>
<th>EU Functions</th>
<th>+Modules</th>
<th>Species</th>
<th>Institution</th>
<th>Country</th>
<th>Course Organizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>F054/15</td>
<td>Designated Vet</td>
<td>--</td>
<td>M, R</td>
<td>Univ Copenhagen</td>
<td>Denmark</td>
<td>A. Hansen</td>
</tr>
<tr>
<td>F025/08</td>
<td>A, B, C, D</td>
<td>--</td>
<td>M, R</td>
<td>Univ Helsinki</td>
<td>Finland</td>
<td>N. Kemppinen</td>
</tr>
<tr>
<td>F11/05</td>
<td>A, B, C, D</td>
<td>-</td>
<td>M, R</td>
<td>Toulouse Vet School</td>
<td>France</td>
<td>M. Kolf-Clauw</td>
</tr>
<tr>
<td>F051/15</td>
<td>A, B, C, D</td>
<td>--</td>
<td>M, R, Z</td>
<td>Univ of Crete</td>
<td>Greece</td>
<td>M. Pavlidis</td>
</tr>
<tr>
<td>F056/16</td>
<td>A, B, C, D</td>
<td>-</td>
<td>M, R</td>
<td>Univ. of Athens</td>
<td>Greece</td>
<td>I. Dontas</td>
</tr>
<tr>
<td>F048/16</td>
<td>A, D</td>
<td>10, 20, 22</td>
<td>M, R, Rb, D, C</td>
<td>Univ Dusseldorf</td>
<td>Germany</td>
<td>M. Sager</td>
</tr>
<tr>
<td>F052/15</td>
<td>A, B, C, D</td>
<td>--</td>
<td>M, R, F</td>
<td>Tanuvas Univ</td>
<td>India</td>
<td>S. Ramesh</td>
</tr>
<tr>
<td>F023/09</td>
<td>A, C, D</td>
<td>10, 20, 21, 22</td>
<td>M, R</td>
<td>Fondazione Santa Lucia Rome</td>
<td>Italy</td>
<td>C Riviello</td>
</tr>
<tr>
<td>F002/03</td>
<td>A, B, D</td>
<td>-</td>
<td>Choice of species</td>
<td>Univ Autonoma de Barcelona</td>
<td>Spain</td>
<td>P. Vergara</td>
</tr>
<tr>
<td>F004/03</td>
<td>LAS specialist</td>
<td>--</td>
<td>--</td>
<td>Univ Autonoma de Barcelona</td>
<td>Spain</td>
<td>P. Vergara</td>
</tr>
<tr>
<td>F026/09</td>
<td>A, B, C, D</td>
<td>20</td>
<td>M, R</td>
<td>Univ of Uppsala</td>
<td>Sweden</td>
<td>M. Le Greves</td>
</tr>
<tr>
<td>F027/08</td>
<td>A, C, D</td>
<td>10, 20, 22</td>
<td>M, R</td>
<td>Univ of Zurich</td>
<td>Switzerland</td>
<td>P. Bugnon</td>
</tr>
<tr>
<td>F030/10</td>
<td>A, C, D</td>
<td>-</td>
<td>Choice</td>
<td>Univ of Oxford</td>
<td>UK</td>
<td>M. Berdoy</td>
</tr>
</tbody>
</table>

C: Cat
D: Dog
F: Fish
M: Mouse
R: Rat
Rb: Rabbit
Z: Zebrafish

FELASA has two accreditation programs:

Accreditation for education and training in laboratory animal science

Accreditation for health monitoring programmes and testing laboratories involved in health monitoring

Following EU-Directive 2010/63/EU and the Working document on the development of a common education and training framework to fulfill the requirements under the Directive (Brussels, 19-20 February 2014), FELASA is accrediting courses using the 'Functions' system', which replaces the 'Categories' system'.

The applicant must have completed and passed the *Laboratory Animal Science EU Function ABD* (former *FELASA type C*) course.

The FELASA Accreditation Scheme is intended for courses that educate and train persons for Functions A, B, C & D defined in article 23 of EU Directive 2010/63, i.e.:

A: carrying out procedures on animals  
B: designing procedures and projects  
C: taking care of animals  
D: killing animals

---

http://www.felasa.eu/accreditation-boards/accreditation-board-for-education-and-training1/
The Danish Veterinary and Food Administration (DVFA) is part of The Ministry of Environment and Food.
The Animal Experiments Inspectorate

The use of animals for experiments, which is expected to be associated with pain, suffering, anxiety or lasting harm is legal only with permit from The Animal Experiments Inspectorate.

Applications to perform animals experiments in Denmark must be submitted through the online application system.

Secretariat and board members

External links

Contact us

AIRD

Offentliggørte tilladelser

NemId

Login med NemId

Parisa Gazerani
Aalborg Universitet
Fredriks Bajers Vej 7A2
9220 Aalborg Øst

Sagsnr.: 2017-15-0201-01197/MABJE

Dato: 11-05-2017
Vedrørende dit tilladelsesnummer: 2017-15-0201-01197

Rådet for Dyreforsøg har på sit møde den 27. april 2017 truffet følgende afgørelse:

Tilladelse til dyreforsøg


Tilladelsen er givet som beskrevet i den vedlagte ansøgning, herunder 1 C-skema.
EXPERIMENTATIONS ON ANIMALS

• You must have a License! Everybody involved must be qualified (FELASA)

• You should make a balance:
  • On one hand to protect the animals
  • On the other to make animal experimentation possible.

• You must plan thoroughly and obtain all the necessary permissions

• Follow all applied regulations

Summary: Ethics in animal experiments

1. **What is preclinical evaluation?** Series of tests and evaluations to make sure that the potential new drug or device is safe and effective.

2. **Why we need to do it?** To avoid safety issues in later clinical uses of drug or medical device.

3. **When we do it?** In drug and device development prior to human use.

4. **How we do it?** Based on guidelines available from FDA or EMEA, following Good Practice.

5. If we do preclinical tests in animals, how we apply **Animal Ethics?** Based on guidelines and legislations (Danish, European Union)

6. **How to Apply Legislations for Experimentations on Animals?**
   - Certificates following courses, plan and design, preliminary tests, real tests, end with reports (Basel, ARRIVE, 3Rs).
Please return in 10 min!
What are Biomarkers?

Kyle Strimbu and Jorge A. Tavel, M.D.
Division of Clinical Research, National Institute of Allergy and Infectious Diseases National Institutes of Health, Bethesda, MD
• A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological response to an intervention.

• Investigative markers are those whose relationship to disease is not clearly established
• Diagnostic markers classify diseased or non-diseased
• Burden of disease markers assess the severity or extent of disease
• Prognostic marker predict future onset of disease
• Efficacy of intervention markers provide information about treatment effectiveness

https://fnih.org/what-we-do/biomarkers-consortium
BLOOD GLUCOSE: A POTENTIAL BIOMARKER

• Glucose level is easily and reliably measured

• Diagnostic marker - elevated glucose indicates diabetes (there are well defined normal and pathological ranges)

• Efficacy of intervention - glucose can be used to monitor treatment effectiveness (administration of insulin or oral hypoglycemics reduce glucose level)

• Prognostic marker – elevated fasting glucose (6.1-7.0 mM) or impaired glucose tolerance 2 hours post glucose challenge (>7.8, <11 mM) is an indication of risk for developing diabetes

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Type 1</th>
<th>Type 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine glucose (UT)</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Blood glucose (UT)</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Hba1c (target)</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Insulin (UT)</td>
<td>↓</td>
<td>↔</td>
</tr>
<tr>
<td>C-peptide</td>
<td>↓</td>
<td>↔</td>
</tr>
<tr>
<td>Antibodies (sel.)</td>
<td>↑</td>
<td>Normal</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>Normal</td>
<td>↑</td>
</tr>
<tr>
<td>Age</td>
<td>Flat incidence</td>
<td>Old</td>
</tr>
<tr>
<td>BMI</td>
<td>Normal</td>
<td>↑</td>
</tr>
<tr>
<td>pH/HCO3 (UT)</td>
<td>↓ (severe)</td>
<td></td>
</tr>
</tbody>
</table>
BENEFITS FROM BIOMARKER UTILIZATION

- Disease understanding
- Project prioritization through early attrition
- Streamlining clinical trials
- Reducing cost of drug development
- Speeding drug development
- Improved decision making
- Avoiding adverse drug reactions
- Rationalizing dosing regimen
- Drug repositioning
Biomarkers

The European Medicines Agency pays close attention to research into the use of **biomarkers** in the **development of medicines**.

Biomarkers are tests that can be used to follow body processes and diseases in humans and animals. They can be used to predict how a patient will respond to a medicine or whether they have, or are likely to develop, a certain disease. For example, the levels of chemicals in the fluid surrounding the brain may be able to predict the likelihood that a patient with mild memory problems will go on to develop dementia due to Alzheimer's disease.

Biomarkers are playing an increasingly important role in the development of new medicines. The Agency expects that their use in research will contribute to **faster public access** to new medicines.

**Activities at the Agency**

On request, the Agency can give an opinion on the **qualification** of the use of a biomarker, to indicate its acceptability for a specific use in pharmaceutical research and development.

For more information, see **qualification of novel methodologies and biomarkers**.

The Agency has also concluded a joint qualification process for biomarkers together with the United States **Food and Drug Administration** (FDA). The qualification followed submission of data by the Predictive Toxicology Consortium (C-Path PSTC) of pharmaceutical companies, and qualified the use of seven biomarkers of drug-induced kidney toxicity in preclinical drug development.
Biomarker Qualification Program

The Biomarker Qualification Program was established to support the Center for Drug Evaluation and Research's (CDER's) work with external stakeholders to develop biomarkers that aid in the drug development process. Through the FDA’s Biomarker Qualification Program, you may request regulatory qualification of a biomarker for a particular context of use in drug development.

Biomarkers can be used in a variety of settings, including basic research, drug development, and clinical practice. The Biomarker Qualification Program focuses on biomarkers used in drug development. Once a biomarker is qualified, it can be used in any drug development program under the context for which it obtained qualification.

The Biomarker Qualification Program is one of the Drug Development Tools (DDT) Qualification Programs created by CDER to provide a framework for development and regulatory acceptance of scientific tools for use in drug development.

Announcement

New! Change in Process for Qualification of Drug Development Tools

The process for qualification of drug development tools is changing under new FD&C Act Section 507. FDA is posting information about these updates to the DDT submission processes.

Check for details, documents and information consistent with new section 507(c).

Learn More

Learn about Biomarkers, their uses in drug development, and qualifying a biomarker

Watch videos about FDA's Biomarker Qualification Program

Explore fictional case studies with “real-world”
Summary: Biomarkers in drug development

- **What?** A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological response to an intervention.

- **Why?** To describe risk, exposure, diagnostic criteria, effects of treatment, investigate biological mechanisms, to predict health outcome.

- **When?** All stages of drug development but stay translatable!

- **How?** Surrogate endpoints, predictors, following guidelines.
WHY WE NEED BIOMARKERS IN ANALGESIC DRUG DEVELOPMENT?

• Current drug treatments for pain either work poorly or are associated with adverse effects

There is a need for novel analgesics that are more effective or safer

• Despite intense research, a lack of real breakthroughs in novel analgesic drug development has seen for decades!! (failure translation from pre-clinic to clinic)
  • We have to recognize that something is fundamentally wrong!
WHAT ARE THOSE BIOMARKERS? WHERE SHOULD THEY COME FROM?

Proper translational biomarkers are biomarkers that can be used from pre-clinical models to the clinic.

- Use same testing methods and biomarkers between animals and humans
- Increase chance of success by aligning preclinical and clinical mechanistic models
- Allows targeting of pain mechanisms for wanted effects
What is a pain bio-marker in drug development?

A technique to provoke pain and pain mechanisms in a standardized way combined with methods to assess the responses quantitatively.
PRE-CLINICAL STUDIES USING MODELS AND BIOMARKERS: HOW?

Thus we need a suitable model and biomarker to:

• Examine the effects of novel analgesic compounds
  • Mechanisms of action, site of action, effect on a disease state, etc
• Predict safety
• Predict failure: which compounds are the most promising to go further in development path?

Pain in humans is subjective, and thus models have been used to attempt to identify objective surrogate biomarkers of pain...
MODELING PAIN IN ANIMALS, HOW?

- Relatively easily modelised
  - Acute pain (both animals and humans)
  - Inflammatory pain (animals > humans)

- Modelised with more difficulties
  - Neuropathic pain (animals > humans*)
  - Visceral pain (animals >> humans, in some cases similarities)

- Impossible to modelise
  - Dysfunctional pain (e.g. fibromyalgia), Complex Regional Pain Syndromes (algodystrophy, causalgia)
  - Interactions with anxiety and depression
## PAIN MODELS IN ANIMALS?

<table>
<thead>
<tr>
<th>Subject</th>
<th>Assay</th>
<th>Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Etiology</td>
<td>Reflex</td>
</tr>
<tr>
<td></td>
<td>• Nociceptive (thermal, mechanical, chemical or electrical)</td>
<td>• Heat or cold</td>
</tr>
<tr>
<td></td>
<td>• Inflammatory (algogen, sensitizing compound, inflammatory mediator, polyarthritic or monoarthritic)</td>
<td>• Mechanical +</td>
</tr>
<tr>
<td></td>
<td>• Neuropathic (surgical or chemical)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Disease state (e.g. cancer, complex regional pain syndrome 1)</td>
<td></td>
</tr>
<tr>
<td>Strain</td>
<td>Body part</td>
<td>Spontaneous</td>
</tr>
<tr>
<td></td>
<td>• Cutaneous</td>
<td>• Autotomy</td>
</tr>
<tr>
<td>Mutant?</td>
<td>• Muscular</td>
<td>• Directed behaviours (biting, flinching, guarding, licking, lifting and shaking)</td>
</tr>
<tr>
<td></td>
<td>• Orofacial</td>
<td>• Gait or posture</td>
</tr>
<tr>
<td>Sex</td>
<td>• Visceral</td>
<td>• Gait or posture +</td>
</tr>
<tr>
<td>Age</td>
<td>• Time point post-injury</td>
<td></td>
</tr>
<tr>
<td>Husbandry</td>
<td></td>
<td>Operant</td>
</tr>
<tr>
<td></td>
<td>• Cage density</td>
<td>• Learned escape</td>
</tr>
<tr>
<td></td>
<td>• Diet</td>
<td>• Place aversion</td>
</tr>
<tr>
<td></td>
<td>• Social factors</td>
<td>• Reinforcement conflict</td>
</tr>
<tr>
<td></td>
<td>Testing procedures</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Arousal</td>
<td>Pain-affected complex behaviours</td>
</tr>
<tr>
<td></td>
<td>• Communication</td>
<td>• Anxiety</td>
</tr>
<tr>
<td></td>
<td>• Handling</td>
<td>• Attention</td>
</tr>
<tr>
<td></td>
<td>• Restraint</td>
<td>• Disability</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Sociability</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Sleep</td>
</tr>
</tbody>
</table>
What type of animal?

- Rodents
- Cheap Translation?
- Cats
- Sleep well (pets)?
- Dogs (pets)?
- Pigs?
- Monkeys?

What type of model?

- Thermal, mechanical or chemical pain (modality) or electrical (non-specific)
- Disease models (inflammation, neuropathic injury, arthritis, cancer)

How do we or even can we measure “pain” in an animal?

- Simple withdrawal (moves away from noxious stimulus)
- Complex behavioral response (lifting paw, vocalization)
### Inflammatory Pain Models

#### Comparison of cutaneous/subcutaneous inflammatory pain models

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Hyperalgesia</th>
<th>Allodynia</th>
<th>Time of onset</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFA</td>
<td>Yes</td>
<td>Yes</td>
<td>2–6 h</td>
<td>1–2 weeks</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>Yes</td>
<td>Yes</td>
<td>1 h</td>
<td>24 h</td>
</tr>
<tr>
<td>Mustard oil</td>
<td>Yes</td>
<td>Yes</td>
<td>5 min</td>
<td>&lt;1 h</td>
</tr>
<tr>
<td>Zymosan</td>
<td>Yes</td>
<td>Yes</td>
<td>30 min</td>
<td>24 h</td>
</tr>
<tr>
<td>Formalin phase I</td>
<td>N/A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>N/A</td>
<td>&lt;1 min</td>
<td>5–10 min</td>
</tr>
<tr>
<td>Formalin phase II</td>
<td>N/A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>N/A</td>
<td>10 min</td>
<td>1 h</td>
</tr>
<tr>
<td>Bee venom</td>
<td>Yes</td>
<td>Yes</td>
<td>1 min</td>
<td>96 h</td>
</tr>
<tr>
<td>Capsaicin</td>
<td>Yes</td>
<td>Yes</td>
<td>1 min</td>
<td>&lt;1 h</td>
</tr>
</tbody>
</table>

<sup>a</sup>Modified and reprinted with permission from the ILAR Journal, 40(3), 1999, Institute for Laboratory Animal Research, The National Academies, 500 Fifth Street NW, Washington, DC 20001 (http://www.national-academies.org/Ilar)

<sup>b</sup>Not applicable
CFA induces significant inflammation in the joint which is more like rheumatoid arthritis. This model responds to TNF alpha sequestering antibodies like infliximab.
Pain Assessment Using the Rat and Mouse Formalin Tests

Nian Gong, Qian Huang, Yuan Chen, Meng Xu, Shuai Ma and Yong-Xiang Wang*

King's Lab, Shanghai Jiao Tong University School of Pharmacy, Shanghai, China

*For correspondence: yxwang@situ.edu.cn
Video 1. The formalin injection procedures and formalin-induced flinch behaviors in the rat and mouse

http://www.bio-protocol.org/e1288
NEUROPATHIC PAIN MODELS

models of neuropathy

• spinal nerve ligation (mechanical)

• streptozotocin treatment (metabolic – induces diabetes)

• taxol treatment (chemical)

ophthalmic (V1) – supraorbital nerve
maxillary (V2) – infraorbital nerve (vibrissae)
mandibular (V3) – inferior alveolar nerve (teeth)
mental nerve (lips chin), lingual nerve (tongue)

Fig. 1. Schematic representation of the spinal cord (A), the exiting spinal nerves (B), and the sciatic nerve (C) with the specific anatomical locations of the nerve injury applied in different models of neuropathic pain in rodents: (1) complete sciatic transection (CST; Wall et al. 1979); (2) chronic constriction injury (CCI; Bennett and Xie, 1988); (3) partial sciatic nerve ligation (Seltzer et al., 1990); (4) spinal nerve ligation (SNL; Kim and Chung, 1992); (5) tibial and sural transection (TST; Lee et al., 2000).
CHOOSE AN ASSESSMENT TOOL?
Hot/Cold Plate NG
The new Hot/Cold Plate NG offers a wide temperature range.

Durham Holders for Orofacial Stimulation
The Durham Animal Holders are new rat holders for trigeminal...

e-VF Electronic Von Frey
The UB Electronic Von Frey of original design, for automatic...

Von Frey Hairs
The Aesthesio® set of 20 monofilaments is based on the Semmes Weinstein...

Orofacial Stimulation Test (Fehrenbacher, Henry, Hargreaves method)
The Orofacial Stimulation Test by Ugo Basile is a new...

P.A.M. Pressure Application Measurement
The P.A.M. (Pressure Application Measurement) device is a novel tool...

Dynamic Plantar Aesthesiometer
The Dynamic Plantar Aesthesiometer was designed to automate the assessment...

Plethysmometer
The first and original device designed specifically to measure paw...

Analgesy-Meter
The 37215 Analgesy-meter is the classic device to perform Paw...

Tail-Flick Unit
The Ugo Basile Tail Flick was designed to measure accurately...

Plantar Test (Hargreaves Apparatus)
The Plantar Test Instrument (Hargreaves’s Method) measures response to infrared...

I.R. Heal-Flux Radiometer
The Hsat-Flux Radiometer Cat. 37300 was designed to calibrate I.R....

Translational imaging
“from MOUSE TO MAN”
STOP
TAKE A BREAK
Use of the Operant Orofacial Pain Assessment Device (OPAD) to Measure Changes in Nociceptive Behavior

Changes in facial expression, coded using a method analogous to facial action coding systems in humans, can be used to evaluate pain in animals.

Several specific changes in facial expression occurred in mice that were in pain, enabling the authors to develop a 'mouse grimace scale' for each of these expressions, and to use this to score the animals' pain.

Administration of analgesics attenuated these changes in expression.

**MEASUREMENTS IN ANIMALS THAT REPLICATE MEASUREMENT IN HUMANS: GRIMACE SCALE**

- The Mouse Grimace Scale (MGS) ranks 5 facial features with the numbers 0, 1 or 2.
  - 0 (not present)
  - 1 (moderately visible)
  - 2 (severe)
BRIEF COMMUNICATION

Nature Methods 7, 447–449 (1 June 2010) | doi:10.1038/nmeth.1455

Coding of facial expressions of pain in the laboratory mouse


Facial expression is widely used as a measure of pain in infants; whether nonhuman animals display such pain expressions has never been systematically assessed. We developed the mouse grimace scale (MGS), a standardized behavioral coding system with high accuracy and reliability; assays involving noxious stimuli of moderate duration are accompanied by facial expressions of pain.
GRIMACE IN HUMANS

• We grimace partly as a communication strategy, to send someone a message: “I’m in pain, take care of me.” In humans, facial expressions of pain can be exaggerated, minimized or faked, although people are also fairly good at detecting it!

• Observations show that similar facial expressions are displayed by neonates and congenitally blind, in response to pain.

• Chronic pain patients (those with pain lasting more than three months) no longer grimace!

FACS - Facial Action Coding System: http://www.cs.cmu.edu/~face/facs.htm
THE FOUR ACTION UNITS OF THE RAT GRIMACE SCALE (RGS)

1. Orbital Tightening
Rats in pain display a narrowing of the orbital area, manifesting either as (partial or complete) eye closure or eye “squeezing.”

2. Nose/Cheek Flattening
Rats in pain display successively less bulging of the nose and cheek (see above), with eventual absence of the crease between the cheek and whisker pads.

3. Ear Changes
The ears of rats in pain tend to fold, curl and angle forwards or outwards, resulting in a pointed shape. The space between the ears may appear wider.

4. Whisker Change
The whiskers of rats in pain move forward (away from the face) from the baseline position, and tend to bunch, giving the appearance of whiskers standing on end.

Sotocinal et al. Molecular Pain 2011, 7:55: http://www.molecularpain.com/content/7/1/55
QUANTIFICATION OF SPONTANEOUS PAIN IN 3 NOCICEPTIVE ASSAYS

(a) intraplantar CFA
(b) intraarticular kaolin/carrageenan
(c) postoperative (laparotomy) pain

Bars represent mean ± SEM RGS score ($n = 6-10$ rats/assay). *$p<0.05$; **$p<0.01$ compared to baseline (Bonferroni-corrected).
A GOOD MODEL CHARACTERISTICS

• replicate the human disease pathogenesis in animals (usually rodents)

• spontaneous or induced disease in animals that mimics human condition

• Positive outcome in the experimental model indicates that the drug will have the same positive effect on the human condition; e.g. drug was effective in an experimental model and also in a clinical setting
  • Sensitivity – ability to predict efficacy
  • Specificity – ability to detect lack of efficacy

• A single animal model will be, at best, predictive of effect for a subgroup of patients.

• Animal pain models are validated by being responsive to a known analgesic (e.g. morphine – visceral pain, NSAID – arthritis)
It may be necessary to use much higher doses in rodents than would be used in man for analgesia.

It is not always determined whether the drug accesses the target tissue or affects the intended receptor target (this can lead to off-target effects).

Modelling disease entities where pathogenesis is poorly understood e.g. most pain conditions.

Many studies measure animal behavior to assess things like pain or stress. Such behavior may be subject to interpretation and vulnerable to bias.

Study design (clinical trials in humans are expected to be well designed, randomized, double blinded to minimize bias) – similar designs are not performed in many animal studies (uncontrolled experimental bias).

Reproducibility and negative unpublished results.

Endpoints of treatment (assay liver tissue in animal but liver enzymes in humans: surrogate).

To overcome: Measurements in animals that replicate measurement in humans.
Summary: Biomarkers in analgesic drug development

- **What?** An animal model or system that can mimic the best of the pain condition at clinic or its pathophysiology e.g. model of trigeminal neuralgia (a type of neuropathic pain)
- **Why?** Unique nature of trigeminal neuralgia, Unique sensory structures (teeth, oral cavity, cornea, etc)
- **When?** Preclinical stage of drug development
- **How?** Nerve injury or ligation in different division
- **Biomarker?** Behavioral (thermal or mechanical sensitivity), nerve activity recording, involvement of non neuronal cells (glial cells) and modulation by drugs to reverse these changes, or preclinical imaging techniques.
- **Imaging measure** dynamic changes over time and yield qualitative and quantitative results that can be used for drug development purposes eg efficacy or safety tests of a drug. Translatable, PET, SPECT, CT, to show anatomical changes, tissue perfusion changes, protein expression, etc.
Animal facility 7H!
REFERENCES

Translational Pain Research
From Mouse to Man
Frontiers in Neuroscience
Edited by Lawrence Kruger and Alan R. Light.
>> Editor Information
Boca Raton, FL: CRC Press; 2010.
Copyright and Permissions

http://www.ncbi.nlm.nih.gov/books/NBK57269/

Animal Models of Pain
Series: » Neuromethods, Vol. 49
Ma, Chao; Zhang, Jun-Ming (Eds.)
A product of Humana Press

REFERENCES

I. Resource: As given by instructors

II. Drug Discovery and Evaluation:
Pharmacological Assays (H. Gerhard Vogel (Ed.), Springer, 2002) available online, free access:

III. The Textbook of Pharmaceutical Medicine, John P. Griffin (Editor)
Case II - group work to practice

• You are working as a consultant for BBB Pharma Company specifically focused on developing analgesic compounds for targeting pain in humans.

• Two weeks from now you have a meeting with the company pre-clinical phase head officer and need to present your animal model to test the efficacy of the new pain killer called AAU007!

• Discuss in your group and make a plan on how would you pick up a model and design tests for efficacy of AAU007.

Questions:

• Which points you will consider the most important ones in selecting a model?

• Do you see some challenges? Where?

• What strategy would you consider to overcome those challenges?
THANKS FOR YOUR ATTENTION