Harm/benefit analysis: cases of research studies using laboratory animals in practice

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Presentation Outline

- Definition of Harm/benefit analysis
- Analysis of Applications
- Analysis of On-going Studies

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Harm/benefit analysis

- **Harm** likely to be caused to the animal
- Potential **Benefit** of the research project

- **Harm**: pain
  suffering
  distress

- **Benefit**: humans
  animals
  environment

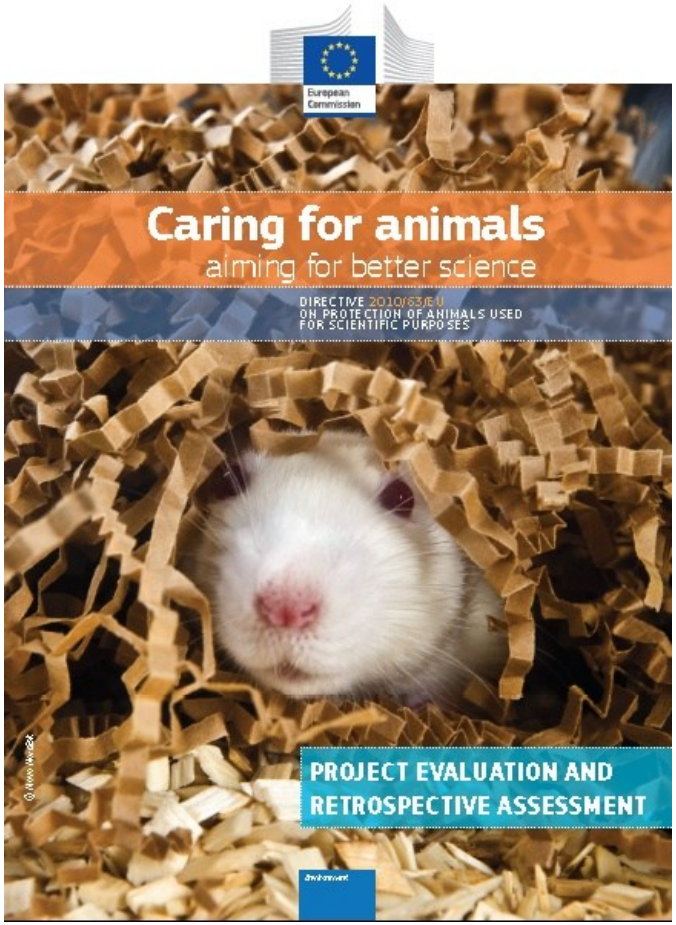
Directive 2010/63/EU, Article 38

Harm/benefit analysis

- Researchers → scientific perspective
- DV → scientific/welfare/legal perspective
- AWB/ERC → welfare perspective
- Project Ev. Com. → scientific/welfare/legal
- Competent Authorities → welfare/legal

Directive 2010/63/EU, Article 38 PE, Article 43 NTPS

Guidance from the EC



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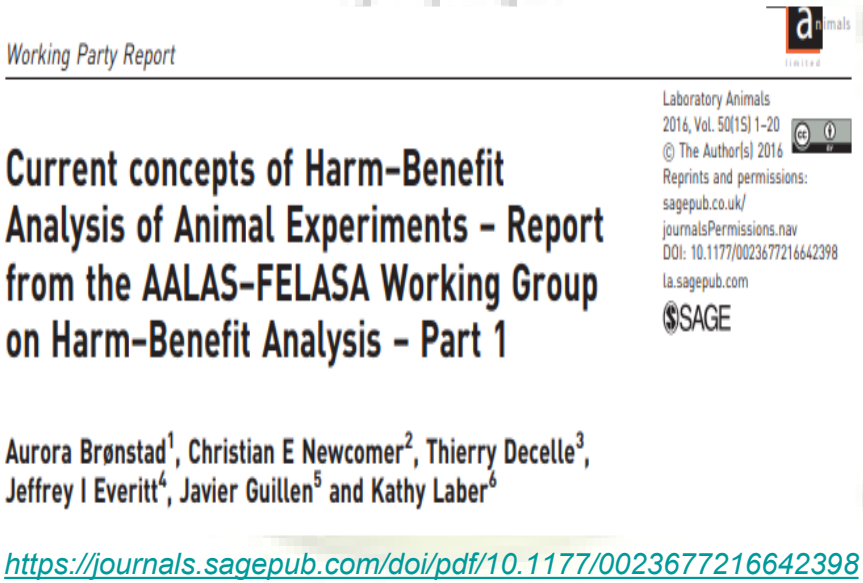
Guidance from a WG



Working Party Report

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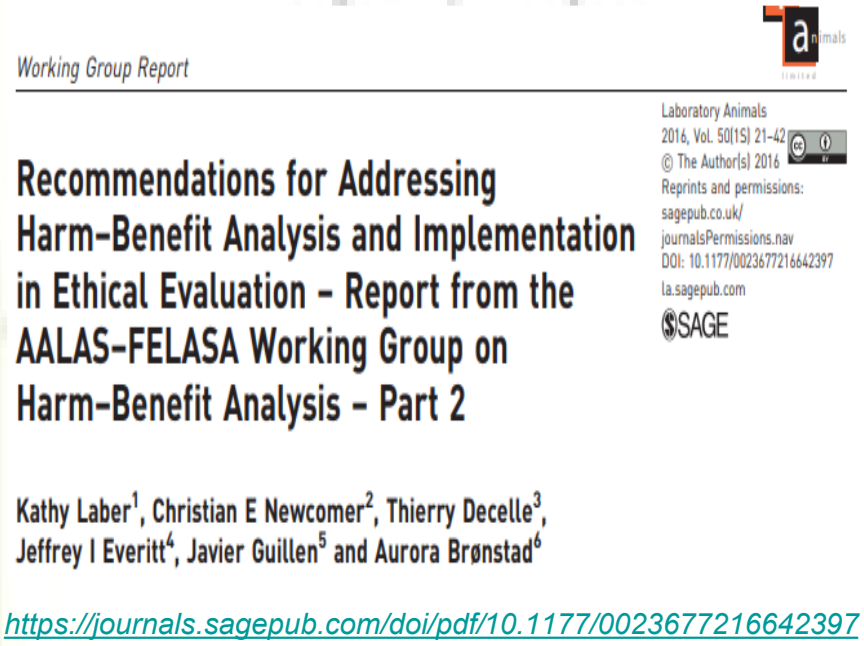
Current concepts of Harm–Benefit Analysis of Animal Experiments – Report from the AALAS–FELASA Working Group on Harm–Benefit Analysis – Part 1

Aurora Brønstad[1], Christian E Newcomer[2], Thierry Decelle[3], Jeffrey I Everitt[4], Javier Guillen[5] and Kathy Laber[6]

https://journals.sagepub.com/doi/pdf/10.1177/0023677216642398



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Recommendations for Addressing Harm–Benefit Analysis and Implementation in Ethical Evaluation – Report from the AALAS–FELASA Working Group on Harm–Benefit Analysis – Part 2

Kathy Laber[1], Christian E Newcomer[2], Thierry Decelle[3], Jeffrey I Everitt[4], Javier Guillen[5] and Aurora Brønstad[6]

https://journals.sagepub.com/doi/pdf/10.1177/0023677216642397

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Harm/benefit analysis of Applications

A) Animals to be used

B) Study information

Harm/benefit analysis of Applications

A) <u>Animals</u> to be used

Number of animals per group

Number of groups

B) <u>Study</u> information

Materials and methods

Procedures (frequency and severity of those to be carried out)

Time plan, etc.

Harm/benefit analysis of Applications

- Potential for **Reduction** – imperative to have a power analysis, ideally by a biostatistician

- Potential for **Refinement** – focus on animal welfare from
- the beginning (source, strain, transport, housing)
- during the project (handling, procedures)
- to the end (humane endpoints, euthanasia)

Harm/benefit analysis of Applications

Important to be updated on the 3 Rs:

- Literature / internet search
- Systematic reviews / Meta-analyses
- Sources of Ethics, Alternative Methods
- Websites of organisations (ECVAM, NC3Rs)
- Discussion with experienced colleagues
- Recognize conflicts between **Reduction** and **Refinement** in the re-use of animals

Harm/benefit analysis of Applications (Case 1)

- Postmenopausal osteoporosis study to be conducted in 3 groups of 8 female rats each: Control, Ovx, Ovx + therapy, for 6 months
- Measurements at 0 – 3 – 6 months:
  - blood biochemical bone markers
  - bone mineral density
- Measurement at 6 months:
  - bone strength (by 3-point-bending *ex vivo*)

Harm/benefit analysis of Applications (Case 1)

- Points to consider:
- Is the control group necessary?
- Could each rat be the control of itself?

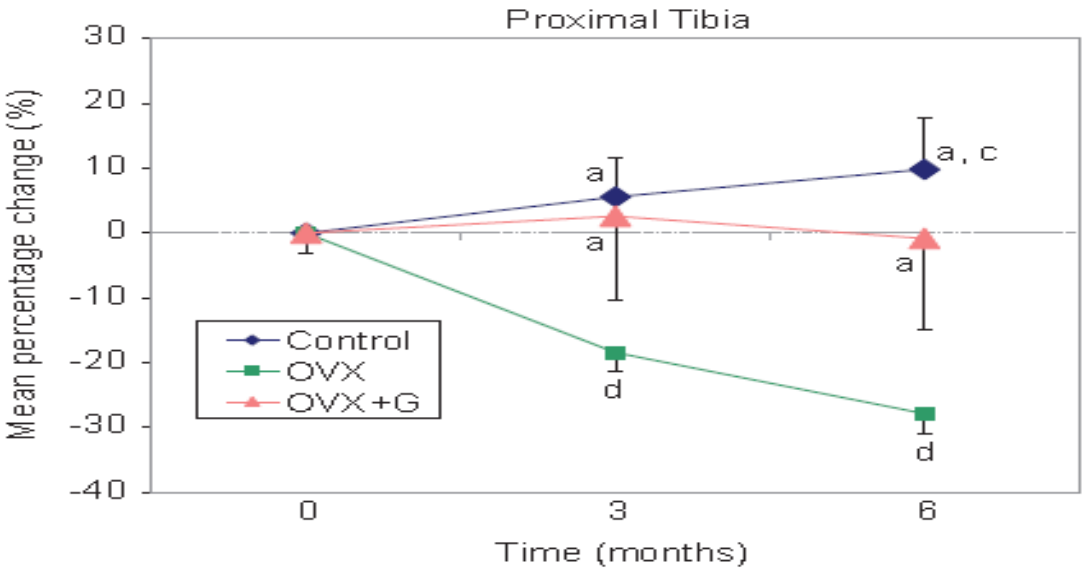

Fig. 3 - Graphical presentation of the mean percentage changes of proximal tibia BMD from baseline to 3 and 6 months of the three groups a: $P < 0.001$ *vs.* OVX, c: $P < 0.05$

Harm/benefit analysis of Applications (Case 1)

- Points to consider:
- Is the control group necessary?
- Could each rat be the control of itself?
- Are the measurements at 0 – 3 – 6 months scientifically justified or could one be cancelled?
- Is the 6-month duration justified as appropriate for an osteoporosis study?

Harm/benefit analysis of Applications (Case 2)

- Study of dietary deficiency of a specific protein, necessary for neural system integrity, on brain enzyme activity and antioxidant status in young lactating rats
- The rats are to be sacrificed by decapitation for blood collection and brain harvesting at age 21 days
- The applicant writes in the application that the use of anaesthesia for euthanasia would render the measurements inaccurate

Harm/benefit analysis of Applications (Case 2)

- Points to consider:
- Annex VI of the Directive 2010/63/EU states:

  Decapitation: (12) “Only to be used if other methods are not possible”
- What does the national legislation permit?
- Is the applicant providing scientific justification of the necessity of decapitation?
- Is the applicant describing the procedure in detail taking animal welfare (harms) into account?
- Is the applicant competent to carry it out?

Harm/benefit analysis of Applications (Case 3)

- Education & Training Course LAS EU F C
- Each Trainee is to be trained in practicals regarding handling and minimally invasive procedures not requiring anaesthesia
- Each Trainer will demonstrate the procedures on one rat and one mouse
- Each Trainee will be trained on one rat and one mouse

Harm/benefit analysis of Applications (Case 3)

- Points to consider:
- Potential reduction of the number of animals to be used
- Harm to the animals in terms of stress during handling, and pain during the minor procedures
- Could each Trainee be trained on one animal species only, i.e. either a rat or a mouse?
- Could each rat or mouse be used for the training of 2 Trainees?

Harm/benefit analysis of Applications (Case 4)

- Study on osseointegration of dental implants in rabbit mandibles
- Each rabbit will be implanted on one side of the mandible – the other is considered the “control”
- 2 types of implants are to be tested (2 x n)
- 2 periods of time are planned for euthanasia and histology (2 x 2 x n)

Harm/benefit analysis of Applications (Case 4)

- Points to consider to ↓ the n of animals:
- Should each rabbit have only one type of implant on one side of the mandible?
- Should each rabbit have two types of implants both on one side of the mandible?
- Should each rabbit have two types of implants, each on either side of the mandible?
- Is it necessary to have a control side or group of rabbits?
- Is there scientific justification supporting the 2 euthanasia time-points? Could only 1 be chosen?

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Harm/benefit analysis of On-going Study (Case 1)

- Experimental atherosclerosis study with 3 groups of 8 Apo E -/- mice:
  A (normal diet)
  B (lipid-enriched)
  C (lipid-enriched + potential therapeutic drug)
- 3 months duration, only monthly blood samplings
- During the 2nd month, 2 mice from group B and 1 from group C developed symptoms of respiratory infection

Harm/benefit analysis of On-going Study (Case 1)

- Points to consider:
- Should antibiotic therapy be given to them?
- Should the 3 animals be withdrawn from the study (and euthanized?) and replaced with new additional mice?
- Will new mice from a different litter have different baseline biochemistry values from those of the original groups?
- Should all mice be replaced?

Harm/benefit analysis of On-going Study (Case 2)

- Experimental diabetes was induced in normal C57Bl/6 mice by streptozotocin i.p. injections
- Fasting blood glucose levels declined in 25% of the mice
- The Researcher desired to repeat injections in those mice (not programmed in the original application), and to delay the onset of the main study in those mice, until they developed increased blood glucose

Harm/benefit analysis of On-going Study (Case 2)

- Points to consider:
- Harm to the animals in terms of pain (additional injections and blood samplings)
- Scientific validity of manipulating the disease model in part of the mice of the study
- Should the mice that did not develop high blood glucose be replaced with new mice?

Harm/benefit analysis of On-going Study (Case 3)

- A study on the effect of drug X on testicular torsion was studied in 8 Wistar rats
- The torsion was on one testis per rat, for 90 minutes, after which de-torsion was applied
- Ischemia/reperfusion injury would be evaluated by pathology of both testes 24 hours after the de-torsion
- The Researcher had an emergency call to Surgery and the 90 min torsion time was extended to 120 min in 2 rats

Harm/benefit analysis of On-going Study (Case 3)

- Points to consider:
- The longer torsion time was not in the Application and Permit
- What should be done with the 2 rats that had a longer torsion time?
- Because of their small number, should their results be deleted?
- Should their results be kept as additional info?
- Should they be replaced with 2 additional rats?

Conclusions

- Harm/benefit analysis of Applications and during projects can identify potential improvements and reject certain procedures based on the **3 Rs**

- **Reduction** and **Refinement** are usually the main issues to consider

- **Refinement > Reduction**

Thank you for your attention