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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]

Abstract

International regulations and guidelines strongly suggest that the use of animal models in scientific research should be initiated only after the authority responsible for the review of animal studies has concluded a well-thought-out harm–benefit analysis (HBA) and deemed the project to be appropriate. Although the process for conducting HBAs may not be new, the relevant factors and algorithms used in conducting them during the review process are deemed to be poorly defined or lacking by committees in many institutions. This paper presents the current concept of HBAs based on a literature review. References on cost or risk benefit from clinical trials and other industries are also included. Several approaches to HBA have been discovered including algorithms, graphic presentations and generic processes. The aim of this study is to better aid and harmonize understanding of the concepts of 'harm', 'benefit' and 'harm–benefit analysis'.

Keywords

harm–benefit, ethical review, animal experiment

Introduction

The growing body of international agreements, regulations and guidelines pertaining to the use of animals in research emphasizes that such use is a privilege granted by society to the research community to facilitate scientific advancement under the condition that animal use is necessary, and conforms to principled and effective animal welfare procedures.[1–4] Use of animals in research is generally accepted by policy makers through regulations, and is based on the presumption that harm–benefit analysis (hereafter HBA) warrants such use. The framework of acceptance has been described in the regulations of the EU Directive 2010/63[2] and in the guidelines described in the *US National Research Council Guide for the Care and Use of Laboratory Animals*, 8th edition (hereafter *NRC Guide*).[1] Typical requirements that must be met when using animals include application of the 3Rs (see Box 1),[5] and some judgment of the likelihood that the outcome of each project will contribute to the core scientific information that ultimately produces benefits and offers the prospect of sustaining or enhancing human and animal lives, as well as protecting the earth's ecosystems.[2]

The need to perform an HBA has been explicitly mentioned in EU Directive 2010/63,[2] the Office International des Epizooties (OIE) *Terrestrial Animal Code*[4] and the Council for International Organizations of Medical Sciences–International Council for Laboratory Animal Science (CIOMS–ICLAS) *International Guiding Principles for Biomedical Research Involving Animals*,[3] and is implied in the *NRC Guide*.[1] The Association for Assessment and

[1]University of Bergen, Department of Clinical Medicine, Bergen, Norway
[2]AAALAC International, Frederick, MD, USA
[3]Chief Veterinary Officer, Sanofi, Marcy l'Etoile, France
[4]Department of Laboratory Animal Science, GlaxoSmithKline, Research Triangle Park, NC, USA
[5]AAALAC International, Pamplona, Spain
[6]Chief, Comparative Medicine Branch, NIEHS/NIH, Research Triangle Park, NC, USA

Corresponding author:
Aurora Brønstad, University of Bergen, Vivarium–Haukeland Hospital, 5021 Bergen, Norway.
Email: aurora.bronstad@uib.no

Box 1. The 3Rs of Russell and Burch.

Replacement: Substitution for conscious living higher animals of insentient animals, or methods not involving animals.
Reduction: Reduction in the numbers of animals used to obtain information of a given amount and precision.
Refinement: Decrease in the incidence or severity of inhumane procedures.
Source: Russell WMS and Burch RL. *The principles of humane experimental technique*. London: Methuen, 1959.

Box 2. Terms of reference of the AALAS–FELASA working group on harm–benefit analysis.

1. Review the existing literature on harm–benefit analysis.
2. Define and describe the current concepts and elements of harm–benefit analysis.
3. Recommend how it can be addressed by persons responsible for the protocol/project applications.
4. Define how harm–benefit analysis can be implemented by responsible entities as part of the ethical evaluation.
5. Present practical cases that may exemplify common situations in the research environment.

AAALAC International has communicated its expectation to all programs participating in accreditation that an HBA based upon the EU Directive should be performed at least in some instances, and underlined advice in the *NRC Guide* indicating that 'the IACUC is obliged to weigh the benefits of the study against potential animal welfare concerns'.[1] More widely, 178 countries participated in the World Assembly of Delegates to the World Organization for Animal Health (OIE) which recently revised the *Terrestrial Animal Health Code* to include Chapter 7.8 (Use of animals in research and education) under Section 7 (Animal welfare).[4] Chapter 7.8 emphasizes the importance of HBA and anticipates inclusion of HBA in the national guidelines of signatory countries by agreement, although there is no legal mandate that would compel them to do so. The European Science Foundation explicitly states that the use of animals must be based on an HBA in a policy document.[6] Also, the *International Guiding Principles for Biomedical Research Involving Animals* have recently been revised in a partnership between CIOMS (which is sponsored by the World Health Organization and UNESCO as well as 170 other international scientific organizations) and the ICLAS (which also enjoys broad international representation).[3] Although this document is also not legally-binding, it serves as a further widely used influential reference reinforcing a globally unified front on the importance of the HBA approach in scientific research using animals.[7]

Both the USA and Europe have designated responsible entities (institutional/regional/national animal ethics committees (AECs) or institutional animal care and use committees (IACUCs)) and charged them with responsibility for project evaluation including HBA.

HBA is based on an ethical stance that expects each scientific endeavor involving research animals to be planned and executed so that harms to animals are minimized and potential benefits from animals are maximized.[8]

The use of animals in research raises ethical questions on the subject of harm, the research objectives and the targeted recipients of the benefit. Animal experiments provide essential knowledge that cannot be achieved by alternative methods, and consequently using some animals to maximize utility for humans, other animals or the environment is inevitable. However, the fact that animal experiments are often useful and essential does not justify the use of animals without ethical qualification in all cases. Historical success of animal experimentation is not sufficient to justify continued animal use, as science is constantly evolving and alternative methods can become available. A justification cannot be reasonably applied as a universal law or as a tool for sweeping and categorical acceptance; as some evaluation of a particular case is expected by the scientific community and by the public where there is a potential for animal harm. Case-by-case evaluation is common practice[9] and not only because it is anchored in regulations and guidelines.[1,2] People apply harm–benefit information in decision-making whether or not they are in favor of a particular type of animal experiment and this applies whether they have positive or negative attitudes to animal experiments.[10] However people still vary in their understanding of HBA principles and will therefore likely apply them differently.

The aim of the American Association for Laboratory Animal Science–Federation of European Laboratory Animal Science Associations (AALAS–FELASA) working group (WG) on harm–benefit analysis is to promote common understanding of the principles and approaches to HBA as an important element in the ethical evaluation of the use of animals in the USA[1] and Europe.[11] Such common understanding and practices might also build confidence in data exchange and collaboration on animal research.

Terms of reference were defined for the AALAS–FELASA WG on HBA (see Box 2). This paper presents the results of tasks 1 and 2, i.e. to review the existing literature and define and describe the current concepts and elements of HBA.

This presentation is based upon the main findings in the literature reviewed of relevance to HBA and the approaches used to systemize HBA and reflect the current understanding of harm, benefit and HBA.

Methods – review of existing literature on HBA

The literature search included publications on harm–benefit or cost–benefit evaluations of the use of animals in research, education and testing. We also included some material on cost/harm/risk–benefit analysis from human medical trials[12] as well as studies on risk–benefit perceptions in general.[13–15] Guidelines and policy statements on the use of animals in research and education (by for example CIOMS, ICLAS, OIE, US Government, European Commission, FELASA and AALAS) were also reviewed.

Results – findings

In the following section we describe how harm and benefit have been characterized and summarize the methods used to compare and weigh harms and benefits.

The cost–benefit evaluation was discussed by Bateson in 1986 in connection with animal research when he introduced the 'Bateson cube' as a model to illustrate the concept.[16]

The term 'cost' has been rejected by some authors as it evokes negative associations with economic cost, and 'cost' has therefore been replaced by 'harm' to make it clearer that it is the negative impact for the animals that is relevant in the ethical evaluation of animal experiments.[7,23] Furthermore, in economic discussions, 'cost' and 'benefit' can be measured in a common currency which has no parallel in research animal studies. In animal studies the subjects potentially experience harm measured in the currency of pain and distress, and the potential benefits, which are often difficult to measure, redound to a different set of individuals (or species) in another category. Comparing apples and oranges is another metaphor used recently to illustrate the difficulty of comparing these two different concepts.[24]

The use of the term 'risk–benefit' appears to be disappearing in the ethical review of animal experiments, compared with references to 'harm–benefit' in human studies. The willingness to take a risk seemingly acknowledges the patient's recognition of a potentially accrued benefit, thus diminishing the contrast of the coupling, 'risk–benefit'. We found the ideas from several human medical trials[12,25,26] to be very interesting and worthy of inclusion in our discussion of HBA in animal studies. This also applies for risk–benefit evaluations in other fields.[13–15,27] In the end there will be people evaluating information and making decisions.

Harm

The WG reviewed the available literature on HBA in animals and key documents on the subject of harm. Different domains or factors that may impair animals and which are relevant to the consideration of harm were identified. Literature suitable to the construction of a helpful framework for a systematic HBA were also selected. The different 'harm' factors that were identified are summarized in Table 2. The harm factors can be subgrouped as 'animal welfare harms', 'animal rights[28] harms/intrinsic nature harms' and 'quality harms', where 'animal welfare harms' is the largest subgroup as shown in Table 2.

Harm caused by painful procedures has been a main concern among antivivisectionists and is one major concern in public opinions of animal experiments.[10] Pain and other impacts of nociceptive physiological processes can also have detrimental consequences for research and the validity of data. However, clinical advances increasingly allow pain and nociceptive responses to be well controlled by the use of appropriate anesthetics and analgesics. Although relevant and important, pain is not the only potential source of harm. Other factors can impact animal well-being negatively by inducing suffering and distress, warranting inclusion in the HBA discussion.

The five freedoms[29] (see Box 4) encompass the impacts on animals in a broader perspective that are more aligned with modern regulations, guidelines and the layperson perspective than only the consideration of pain.

These include not only harm caused by pain, but also any aspect that can compromise animal well-being, including the opportunity to express normal behavior. The five freedoms were originally defined for farm animals,[29] and Mellor and Reid can be credited with adapting the five freedoms to discriminate between harm levels in research animals.[30,31]

Injuries and diseases are inherent harm factors relevant to many animal experiments. The negative impact of these can often be minimized or controlled by different refinements that mitigate the negative effect on animals such as the use of analgesics in the case of pain. Experimental conditions can also cause fear, anxiety and distress for animals and are also legitimate harm factors. Minor procedures alone might not cause a significant negative impact; however if they are repeated frequently or the procedure lasts for a longer period or conducted over a substantial part of the animal's lifespan, then the total burden must be regarded as harmful for the animal. Frequent transport, single housing of social animals and impeding an animal's ability to express normal behavior are examples of such harm factors. Transport of animals over shorter distances is not regarded as a major impact per se, but the stress of frequent transport requiring the animal to continually adapt to new locations would warrant consideration. Animals need time to rest and express normal behavior,

Box 3. Background and Impetus for harm benefit analysis.

The idea that HBA should precede experimentation on live subjects was originally developed in relation to experimentation on human subjects. The importance of considering the harms and benefits in relation to experimentation was first introduced in the 10 principles of the Nuremberg Code[17] developed following the Nuremberg trials on human experimentation after World War II. The Nuremberg Code emphasizes that experiments should be such as to yield fruitful results for the good of society, unprocurable by other methods or means of study, and not random and unnecessary in nature. The Nuremberg Code also provided that experiments should be so conducted as to avoid all unnecessary physical and mental suffering and injury and experiments should assure that the degree of risk to be taken should never exceed that determined by the humanitarian importance of the problem to be solved by the experiment. Notably, the Nuremberg Code identified animal studies as a crucial first step in the protection of humans from the primary exposure to risks by establishing a results-based scientific foundation for the belief that similar anticipated results in humans will justify the performance of the experiment. Subsequent to the Nuremberg Code in the late1970s, the Belmont Report[18] in the United States and the World Medical Association Declaration of Helsinki[19] elaborated ethical principles for the conduct of human studies sharpening the concept that the harms and risks to human subjects participating in experiments should be evaluated in relation to the benefits accrued from participation. Quoting Principle 18 of the Declaration of Helsinki, 'Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them'[19] – interestingly similar principles were not then specified for experiments in animals.

There is a longstanding precedent for the use of animals in research for the pursuit of outcomes beneficial for humans or animals. In 1985 international principles were developed by CIOMS offering guidance to governments and scientists in countries with a broad spectrum of regulatory oversight mechanisms.[20] Concomitantly very similar principles were adopted by the US Government,[21] and in Europe in the preamble of the European Treaty Series (ETS) 123.[22] In both cases the principles embraced the notion that studies in living animals should be designed and performed with due consideration of their relevance to human or animal health and the advancement of knowledge while the US guidelines also added animal studies for 'the good of society' as a motivation with merit.[21] Both sources suggested that where one or more of the guiding principles potentially would be compromised by the nature of the animal study, an appropriate responsible entity or Animal Ethics Committee (AEC), rather than the investigator directly concerned with the studies, should have decision authority and that exceptions to the guidelines should not be made for teaching or demonstration purposes.[21,22] The CIOMS statement and the US Guiding Principles statement on this are almost identical and are already summarized in the text provided. In 1985 CIOMS stated 'VIII. Where waivers are required in relation to the provisions of article VII, the decisions should not rest solely with the investigators directly concerned but should be made, with due regard to the provisions of articles IV, V, and VI, by a suitably constituted review body. Such waivers should not be made solely for the purposes of teaching or demonstration'[20]

These guidelines served as the impetus for the later development of the HBA concept by institutional/regional/national AECs or IACUCs. However, while broadly inferring the importance of benefits, the 1985 guidelines did not specifically elaborate on the scope and kinds of benefits that might be deemed relevant. In recent years, regulatory guidelines as well as an interested public have sought greater clarity and precision in the disclosure/discussion of the benefits perceived from animal research, and public support and financial support for science would be well served by improving our efforts in this area. The guidelines were not instructive in 1985 about what constitutes a benefit and there was no additional clarification in the guideline revisions in recent years. The concept of a benefit seems to be quite broad and defining it is left to the bodies overseeing the research. The key change in the old versus the new guidelines is that the early guidelines indicated that harm should be reduced and science should be conducted for a relevant and productive purpose. Later guidelines suggested (or imposed) that committees should link and measure the harm–benefit relationship.

The United States and Member States of the EU that support research animal use have defined systems or bodies (e.g. AECs, IACUCs and other responsible entities such as national committees) that are charged with the responsibility for ensuring that animal use is in accordance with regulations and ethical norms and authorizing projects based on specific information in applications provided by the scientist conducting the research animal studies. A summary of similarities and differences for project evaluation and authorization in the USA and EU is presented in Table 1. Regulatory compliance is not just important to the responsible entities overseeing research. Compliance with regulations and operating within a consistent ethical framework are important priorities for scientists who are constantly pursing the simplest experimental model systems that yield rigorous and reliable data. Operating without a consistent ethical framework may damage the reputation of research and increases the risk of negative public perceptions of the specific research activities and the broader research enterprise.

Table 1. Similarities and differences for project evaluation and authorization in the USA and EU.

	US USDA Animal Welfare Act	US PHS Policy and the Guide for the Care and Use of Laboratory Animals	EU Directive
Responsible body for protocol/project evaluation	Institutional Animal Care and Use Committee (IACUC)	Institutional Animal Care and Use Committee (IACUC)	Public Competent Authority As this function can be delegated to other bodies: a number of Member States allow local animal welfare bodies/ethics committees and/or external ethics committees to perform the evaluation
Minimum composition evaluation body	Three members including a DVM and a non-affiliated member	Five members including a DVM, a practising scientist, an expert in non-biological science and a non-affiliated member	Expertise in: (a) the areas of scientific use for which animals will be used including replacement, reduction and refinement in the respective areas; (b) experimental design, including statistics where appropriate; (c) veterinary practice in laboratory animal science or wildlife veterinary practice where appropriate; (d) animal husbandry and care, in relation to the species that are intended to be used
Harm/benefit analysis	–	IACUC is obliged to weigh the objectives of the study against potential animal welfare concerns	A harm–benefit analysis of the project, to assess whether the harm to the animals in terms of suffering, pain and distress is justified by the expected outcome taking into account ethical considerations, and may ultimately benefit human beings, animals or the environment
Responsible body for protocol/project authorization	IACUC	IACUC	Public Competent Authority. Only Belgium allows institutional ethics committees to approve projects that they have evaluated favorably
Other bodies involved	–	–	Local (institutional) animal welfare body (AWB). This includes the person(s) responsible for the welfare and care of animals, a scientist (in the case of users) and input from the designated veterinarian
Post-approval monitoring	Yes, by IACUC	Yes, by IACUC	Yes, AWB to follow the development and outcome of projects
Retrospective assessment	–	–	Yes, at least for all projects using non-human primates and projects involving procedures classified as 'severe'. Assigned to the competent authorities, but it can be delegated to

Table 2. Harm factors identified in literature review.

	Animal welfare harms																		Animal rights harm		Quality harms	
Ref.	Species, choice of animals	Sentience and consciousness	Quality of animals	Duration	Duration related to lifespan	Number of animals	Origin, acquisition or transport	Care, housing factors, handling, health care	Possibility to express normal behavior	Staff competence and quality	Hunger and thirst	Discomfort	Pain, injury or disease	Fear, anxiety and distress	Frequency of procedures	Severity	Risk of harm = probability × severity	Deaths (caused by the experiment)	Intrinsic value and animal rights	Genetic modulation - respect for nature	Aim, realistic potential scientific quality	Non-publishing of negative results
Bateson 1986[16]								+						+							+	
Bateson 1992[37]	+												+	+					+			
Porter 1992[32]	+			+	+	+		+					+								+	
Mellor 1994[30]		+							+		+	+	+	+								
Stafleu 1999[40]	+		+	+		+				+	+		+	+	+				+		+	
Mellor 2004[31]									+		+	+	+	+								
Rickard 2004[56]	+							+		+												
Voipio 2004[7]	+			+		+	+	+	+	+			+	+	+	+		+				+
Schuppli 2004[38]	+	+				+		+	+	+			+	+					+	+		
FELASA 2005[41]						+	+	+		+			+	+		+						
Degrazia 2007[49]							+	+			+		+	+				+				
EU Dir. 2010/63[2]	+					+	+	+					+	+		+		+				
Boddy 2011[26]														+			+					
Lindl 2012[60]													+	+								

Box 4. The five freedoms.

These are currently expressed as:

Freedom from hunger or thirst: by ready access to fresh water and a diet to maintain full health and vigor.

Freedom from discomfort: by providing an appropriate environment including shelter and a comfortable resting area.

Freedom from pain, injury or disease: by prevention or rapid diagnosis and treatment.

Freedom to express (most) normal behavior: by providing sufficient space, proper facilities and the company of the animal's own kind.

Freedom from fear and distress: by ensuring conditions and treatment which avoid mental suffering.

and this can be disturbed by the imposition of frequent procedures.

Individual procedures causing a moderate negative impact can produce a severe negative influence on animals if they are repeated over a long period or if many are compressed into a short time interval.

The duration of such an impact as a proportion of the lifespan of the animal is another relevant domain in this discussion.[32] Cumulative harm reflects the total negative impact of an animal's experience through its whole lifespan.[33] Harm has also been defined as a product of the probability and the severity of harm.[26]

Animals are affected by housing and the care provided. Housing facilities must be suitable to cover the animals' needs, and the facility physical plant must be properly managed to avoid animal injuries. Simple things such as daily handling and care can be experienced as harmful if the staff members responsible are not appropriately qualified. For example, handling of fish often includes taking them out of water, their normal habitat. Being out of water is a life-threatening condition causing severe stress for the fish. The quality of care will depend very much on the competence of the staff in the animal facility, which includes their knowledge, experience, skills and motivation. Their ability to recognize any sign of negative impact on an animal and their ability to take corrective steps will make a significant difference for the animal's experience in an experiment. The concept of care exceeds mere harm avoidance; good animal care also includes proactive actions that optimize animal well-being, as reduction of well-being is a form of harm.

All harm factors mentioned so far are dependent on sentience and the degree to which the animal is aware of its situation. Some animals are regarded as more sentient than others, making the species of animal used a relevant factor in the evaluation of harm. According to the 3R principles, replacement can be 'substitution for conscious living higher animals of insentient animals'.[5] The definition of what constitutes 'conscious living higher animals' is evolving and controversial, inviting comparisons to Singer's discussion of speciesism.[34] Our understanding of the conscious species with the capacity to suffer from harm depends very much on our knowledge of that species. Non-human primates are regarded as 'conscious living higher animals' that need extra attention by some groups. Article 8 in the EU Directive explicitly mentions the use of non-human primates reflecting a stance that doing experiments using non-human primates is regarded as more harmful than using other species. Such a stance can be discussed as some species are more easily habituated to human contact and experimental conditions than others.

Accelerated genetic predisposition to disease and putting an animal at risk of harm by developing a chronic debilitating or devastating disease may cause much distress for the animal, even if pain is not the main issue in the earlier phases of phenotype progression. This applies for some of the genetically-modified animal models that are used.

The number of animals has also been included as a dimension of harm. However, the reduction principle, or limiting the harm to a few, can sometimes be at odds with a refinement effort which results in little harm but involves a greater number of animals.[35,36] Experimental approaches involving cumulative mild harm to many animals in lieu of more severe harm to a few animals create ethical dilemmas that warrant thoughtful analysis and resolution.[35] Failure to respect intrinsic values has also been suggested by some authors as a harm factor.[37–40]

Professional societies have addressed the importance of recognizing harm and pursuing harm reduction in their publications. FELASA working groups[11,41] have extensively documented different experimental procedures and offered an assessment of how these might influence animal well-being. Two AALAS Position Papers[42,43] have also addressed harm and harm reduction in relation to the matter of judicious animal use. But none of these sources offers guidance on calculation of the HBA.

Factors related to the research animal study aim, potential for success, design of experiments and (lack of) publication of results have also been mentioned as harm factors.[16,32,40] Animals are not harmed more by low-quality design than by high-quality design per se. Performing poor-quality experiments that are not likely to yield valuable information is an irresponsible use of animals – independently of whether the animals experience much harm or not. Poor-quality studies can cause harm if they produce misleading results.[44] In the Bateson cube model, quality of design is presented as a separate domain or dimension – independent of harm and benefit, emphasizing the importance of this factor.[16]

Severity classifications and discrimination of harm levels – the need for categories

From the discussion above, it can be difficult to compare circumstances across different harm domains, e.g. comparing minor surgery under anesthesia with extended housing in isolation for a social species. Different ways to evaluate overall harm, e.g. categorizing harm into severity classes,[33,45] have been developed. The baseline of zero (0) harm is vaguely described and can be approximately equated with any procedure comparable with needle injection in Europe.[46] Some authors define categories for each harm domain and on this basis make a cumulative/overall severity/harm score for the experiment.[30–32] Others describe the different categories using examples of procedures.[47] Killing animals for their organs and tissues is not considered a procedure, and thus is not subject to HBA according to European regulations.[2] Anesthetizing an animal without any prior intervention and then euthanizing the animal under anesthesia is classified as a separate harm category under European regulations,[2] although this requires the consideration of the applicability of alternatives (i.e. the 3Rs) for animal species covered by the US Animal Welfare Act regulations. The reason for categorizing this in a separate severity class is that the opportunity for potential negative experiences for the research animal is minimized and is effectively limited to ineffectual anesthesia,[46] even if death is the outcome. This is controversial, and not everyone agrees that this is less severe, even if suffering is not involved,[37] because animals have an intrinsic value.[40]

Maximum severity encompasses conditions that cannot be treated, relieved or involve death as the outcome. Such experiments should always be carefully considered regarding refinement and use of more humane endpoints.

EU Directive 2010/63 demands the classification of experiments according to the level of harm in research animal studies in order to ensure that science is public-accountable.[2] A European Commission expert working group has identified four categories, i.e. terminal, mild, moderate and severe,[33,46] providing guidelines on classification and offering specific examples on how the classification scheme should be applied. Severe experiments include those that have a serious impact on animals for any duration or a moderate impact over a

long time. Severely impacted animals may experience devastating disease or even death as a potential experimental outcome.[33] The United States Department of Agriculture (USDA) has defined pain/distress categories based on whether or not the animal experiences pain or distress; if pain or distress can be relieved with pharmacological interventions; or, the most severe category, experiments where the animal experiences pain/distress but interventions are withheld due to scientific necessity.[48] The USDA Pain and Distress Categories document also includes a guide on how to classify specific experiments.[48]

Harm to animals is generally predictable as long as the experimental plan contains all necessary details of the procedures used in the animal studies.[49] Based on knowledge of how these procedures may impact animals, prospective assessment of harm is possible and actions to reduce or eliminate harm (i.e. refinements) can be implemented. However, unpredictable experimental outcomes sometimes occur, and a retrospective review of an experiment can reveal useful information to aid planning in subsequent experiments of a similar type to avoid or reduce harmful circumstances.

Severity classification of animal experiments is helpful to the responsible entities when planning their review processes. As noted previously, mild procedures that are repeated frequently may also interfere with the animal's ability to perform normal behavior or recover between procedures. Frequency of procedures is therefore relevant. An overview of procedures along a timeline can be schematized using an activity chart/map that records all the procedures for an animal along that timeline.[50]

The new reporting system for animal experiments in Europe includes a classification of experiments.[33] Illustrative examples for the process of severity classification, day-to-day assessment and actual severity assessment are also provided.[47] Declassification of experiments to lower classes is a way of monitoring performance in the refinement of experiments. Refinement is an otherwise complex concept involving technical insights and impact measures that are not as readily communicated to lay audiences as the simple reporting of numbers of animals used (reduction measures).[36]

Classification or quantification of the harm and benefit metrics of an experiment is a possible way of communicating with the public about the use of animals in research. The public is commonly focused on animal studies perceived as involving severe harm; however the majority of experiments are likely to be classified in the moderate or mild harm category. By successful implementation of refinement strategies severe experiments can be avoided.

Benefits

An overview of benefit dimensions identified in the literature reviewed is presented in Table 3. Benefits identified in this review can be divided into three main dimensions: scientific quality, promise or potential outcome, and actual outcome.

Science and society, through regulations, have accepted the use of animals as surrogates for humans and as basic research subjects in diverse scientific endeavors regarded as beneficial to society, especially to improve human health, veterinary medicine, safety testing and to advance scientific knowledge.[8] The use of animals for educational purposes has also been proposed,[7] and a discussion of whether economic interest justifies the use of animals has also been raised.[7,37,40]

In Europe, potential benefits have to be described in a project summary intended for the public according to the consensus document providing guidance for the drafting and publication of non-technical project summaries.[2] A template and an illustrative example have been published.[51] Similar provisions can be obtained in the USA through the checklist of the protocol review delineated in the *NRC Guide*[1] and through Principle II of the *US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and* Training.[21] Researchers need to explain the potential benefits derived from investigations in research animals.

Benefits which can be categorized as 'future promise' may never be realized and it can be difficult to describe them precisely. 'What, who, how and when' have been suggested as key questions to ask to better address what the anticipated benefits are.[52]

Historically animals have been used as models for humans in studies of basic biological/disease mechanisms, product safety evaluations and development of new therapies. They have also been used frequently for studies in environmental/ecological science, agriculture/production enhancement and as models for disease studies in non-human species. These are all examples of benefits that accrue advantages to individuals, groups of individuals or societies at large that require animal use in scientific endeavors. Primary benefits of this nature would include the intrinsic value of knowledge itself and the relevance of this knowledge in applications that are directly beneficial to humans and other species or the global environment and that sustain the quality and diversity of life. Examples of primary benefits also include the impact of potential improvements in cancer therapy for a patient, with cancer targeted by that therapy. There are also examples where fundamental studies in a species can be extended to taxonomically similar invertebrates. The protection of cephalopods in Europe[2] is such an example and is based on studies on that species and recommendations from scientists.[53]

Table 3. Benefit factors identified in literature review.

	Quality dimension				Promise dimension							Actual benefit dimension			
Reference	'Good science' dissemination of results	Originality	Aim and objectives	Realistic potential, feasibility	Benefits for humans	Benefits for animals	Benefits for environment	Economic interests	Health interests	'Surrogate outcomes' versus 'health outcomes'	Secondary (long-term) benefits	Primary (short-term or direct)	Safety interests	Knowledge interests	Educational interests
Bateson 1986[16]	+														
Bateson 1992[37]	+				+	+	+	+						+	
Porter 1992[32]			+	+											
Mellor 1994[30]											+	+			
Stafleu 1999[40]			+	+				+	+	+				+	
Mellor 2004[31]											+	+			
Rickard 2004[56]	+				+	+									
Voipio 2004[7]	+			+	+	+	+	+	+				+	+	+
Schuppli 2004[38]	+														
FELASA 2005[41]	+	+	+	+											
Degrazia 2007[49]					+										
EU Directive 2010/63[2]	+		+		+	+	+							+	
Boddy 2011[26]	+										+	+			
Boyd 2012[25]										+					

In this case, the benefits apply to the class 'Cephalopods' (phylum Mollusca) as a group, rather than to the individual research animal. Some experiments might have benefits applicable to several domains. Experiments directed to improving efficiency in agriculture can have economic interests as the primary aim or benefit. According to Mellor[31] economic benefits alone should not be used to justify animal experiments, especially when they cause much harm. However economic benefits for the farmer by reducing production costs can go alongside benefits for the environment by reducing pollution or by more efficient utilization of commodities, and also with improved health and well-being for the species in question. In some cases such studies can be a benefit for the actual animals involved in the study by providing them with improved conditions to maintain good health and well-being as well, and knowledge gained from these studies may benefit the farmers and stakeholders involved in the farming industry.

The secondary benefits identified are not necessarily dependent upon the data derived from sentient animal models and these benefits should not qualify as sufficient reasons for research in animal models causing harm in the absence of a compelling potential scientific reason. Economic benefit is an example of a secondary benefit. This does not only refer to the economic advantages accrued to a single researcher, but also to the economic benefits to the community built around the industry that commercializes or utilizes the scientific finding(s). For example in the cancer therapy scenario above, significant secondary benefits are the consequences for the health-care system of cost reductions (compared with cost overruns) resulting from new therapies, and/or families' and society's benefits from health improvements in family earners or the workforce generally. Also, the commercial potential derived from findings in animal research studies can result in products that will be available to improve the lives for many people or for animals, thus further increasing the benefit. The term 'wider benefit' has also been used for such secondary benefits.[26] The potential for a high value research outcome (research quality factor) and the likelihood of research success (probability of achievement factor) have been included as valuation domains in the assessment of benefits. The likelihood of benefit will depend on the likelihood of the success of a research project in its methodological approach, data acquired, and data impact or applications.

Other examples of secondary benefits include improved organizational reputation and success related to productive scientific endeavors or enhanced career prospects, earning power, and consequently family benefits for individual scientists and their support staff needs. Secondary benefits can also include enhanced benefits for the wider community (the city, region or country) through economic, educational, social and other contributions made by the organizations, scientists and support staff who are part of the community. Increased recognition of the intellectual arena, the exercise of our creative imagination, rationality and problem-solving skills in the pursuit of the animal-based branch of science, which is part of our culture, are also examples of secondary benefits.

Humans involved as subjects in scientific research are cognizant of the risks. They offer informed consent to proceed with the knowledge that they, others with the condition under study or others of their species will have the prospect of benefiting from the outcome of the research. However, there are some exceptions where subjects are unable to give informed consent such as research in pediatrics, geriatrics, dementia, etc. In all these cases the risk is evaluated by a proxy (parent, guardian or committee) who makes a decision on their behalf. Animals cannot offer informed consent comparable with a competent person (as distinct from positive reinforcement training to accept minor procedures). Therefore the responsible entities must ensure that animal interests are accounted for. Animals enrolled in clinical therapeutic trials for naturally occurring diseases may experience a direct benefit from participating as study subjects. This may happen more often as a result of our improved diagnostic ability for selecting veterinary patients with relevant clinical disorders for use in proof of principle studies with our increasing ability to identify promising molecular targets and evaluate the safety of interventions in non-animal systems. Recent examples of veterinary patients benefiting in this fashion include canine cancer therapy evolving through molecular markers and pathway studies in human tumors and rodent models and regenerative stem cell therapies in companion animals developed through studies in rodents and other model systems.[54,55]

Undoubtedly, the rapidly accumulating progress in our understanding of the genetic and molecular basis of disease and therapeutic response through animal and non-animal model systems will allow more animals to benefit directly through their participation as research subjects in the future as we improve our ability to recruit appropriate target populations for study. However, in most instances animals have been used historically and will continue to be used in research studies or educational and testing applications as proxies for another species and while receiving no immediate direct benefit and with an unknown prospect of receiving a future benefit as part of the species.

As noted above, in some cases animals participate in research projects analogously to humans, without informed consent, that elucidate whether or not a new therapy will benefit them directly as active clinical patients. This type of research animal utilization may become more prevalent as molecular medicine and personalized medicine continue to advance. However, to limit scientific inquiry in animals only to these types of studies would be anathema to the interests of the global scientific community and society and inconsistent with the use of animals for other societal objectives.

Factors related to quality and potential, design of experiments and publishing have also been defined as benefit factors by some authors,[2,7,16,26,37,38,41,56] and poor performances on these can lead to unreliable results or lack of dissemination of results and thereby reduces likelihood of any benefits. Without a plan for dissemination of results, realization or impact of a study is less likely.[26]

Based on the literature we have reviewed it seems that benefits, in contrast to harms, are less well defined with regard to classifications or means to strengthen benefits in operational terms. This can be explained by the fact that performing HBA in a systematic way and thereby defining and describing benefits is not common practice. For harm the 3Rs have been an operational algorithm for reducing harm since 1959.[5] The quality of scientific experiments is definitely a factor that impacts benefit in the way that a well-designed experiment is a fundamental criterion for reliable information and for generating any benefit at all. Actual outcome benefits, like acquiring skills through training or safety testing in animals, have direct application; however in many situations alternative methods are available and the use of animals is therefore not justified. Independent of whether promised or potential benefits are realized – most agree that experiments contribute to increased knowledge and the understanding of a phenomenon. The question is then whether this knowledge is of such importance that animal use is justified.

Balancing and comparing harms and benefits

In the previous sections we have defined and described the current concepts and elements of harm and benefit. We have summarized different dimensions of harm and benefit discussed in the literature reviewed in Tables 2 and 3. An HBA also includes a systematic way to compare and weigh the harms and benefits. In the literature

Table 4. Summary of the strengths and weaknesses of different models of harm–benefit analysis (HBA).

	Strengths	Weaknesses
Categories	Categories are useful for simplifying a complex picture. Identify severe categories and stimulate actions to avoid them.	The categories do not fit all cases.
Algorithms	Algorithms are helpful in guiding a decision.	Moral dilemmas cannot/shall not be solved by arithmetics.
Graphic representations	Graphic representations have pedagogic value in visualizing the concept and relationship between harm and benefit.	Depend on defined categories. Not operational.
Process-oriented models	Process-oriented models structure the HBA process, how to balance different opinions and question quality of the analysis. Generic.	Do not provide an answer on what models to use or provide solutions for conclusions.

reviewed there is a mixture of systems to categorize these parameters in lists of attributes or questions to be addressed in the analysis of harm and benefit. A summary of the models and their strengths and weaknesses are presented in Table 4.

Algorithm models

An algorithm is a process or set of rules to be followed in calculations or other problem-solving operations. Ideally, an algorithm should work in all situations, and providing the 'correct' solution in every case.

Stafleu et al. have presented a complex set of formulas for calculating scores for harm and benefit.[40] This study has distinguished itself from other studies by providing a more complete model for scoring benefits in a systematic way as it has provided a set of formulas.[40] Other mathematical models describing harm–benefit as fractions or as a sum have also been described to illustrate the HBA concept on balancing harms and benefits.[57] However these models did not provide an algorithm on how to estimate the size of harms and benefits. Since there is no common 'currency' or summary metric that reflects harm and benefit in a way that makes it possible to compare these sizes,[37,58] others prefer a non-numeric scale using letters to avoid the apparent and misleading precision of arithmetic assessment.[31] Other authors are not in favor of mathematical models because they give a false impression of objective accuracy.[7]

Mellor[31] has stressed that harm–benefit evaluations cannot be reduced to an arithmetic exercise. In the Mellor et al.[30,31] model, harms are categorized using letters (O, A, B, C and X, where X is the most severe category) instead of numbers. This was done intentionally to avoid any temptation to use arithmetic to draw a conclusion.[30,31] Evaluation in the Mellor's model is made based on the impact of the experiment on each dimension of the five freedoms.[29] The greatest anticipated compromise specific to each domain is used as a final grade. Mellor[30,31] discriminates between primary (or direct) benefits and secondary (or indirect) benefits. Grade A can be justified by both primary and secondary benefits, while B, C and X can only be justified by primary, direct benefits.[31]

Graphic representations

Graphic models help the reviewer visualize the relation between harm and benefit using a graphical illustration such as a figure or a diagram. The first published report of a practical approach to conducting a general HBA in animal research studies can be credited to Bateson in 1986[16] and based upon his work the role of the HBA first appeared in national guidance documents in the UK Animal Scientific Procedures Act in 1986. The UK subsequently incorporated the requirement for an HBA in the ethical review process and several other countries including Norway, Brazil, Tanzania and Australia have since adopted similar provisions.[59]

The Bateson square is an example where harm and benefit are presented along the two axes.[16] Common traffic light colors have been added to indicate the favorable/unfavorable status of a dimension: green means acceptable; yellow signifies attention; and red indicates stop. Bateson also introduced research quality as a factor in the third dimension (z-axis) and thereby introduced a 3D cube model.[16] More complex figures like flow charts and decision trees can be used to include more dimensions.[57] However, as soon as the model becomes too complicated it loses its power as a simple graphic presentation.

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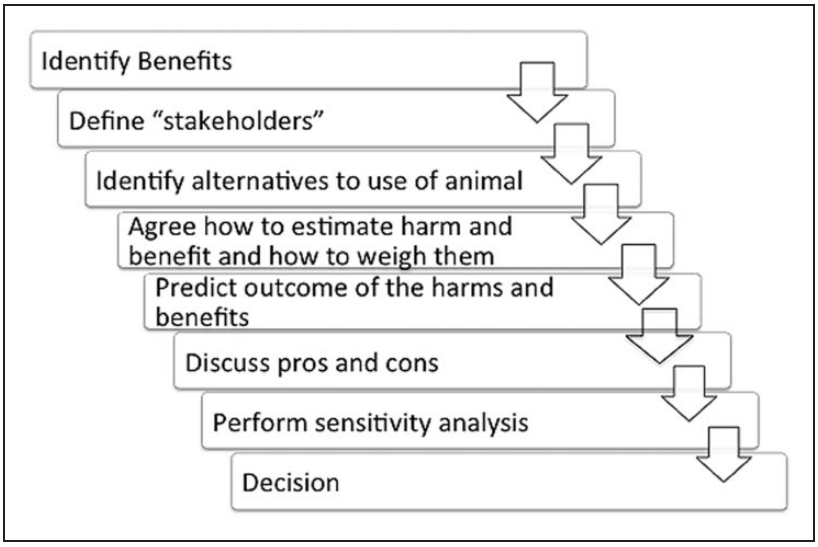

Figure 1. A generic harm–benefit analysis.

has developed a modified Bateson cube.[52] Bout et al. have presented a refined model based on Bateson that has been used for HBA in The Netherlands.[9]

Models like the Bateson model have been widely recognized for their pedagogic value to illustrate the concept of HBA. They are normative, illustrating that high harm and low benefit projects should be rejected. However, they are not necessarily operational so they cannot always be applied productively in challenging situations commonly encountered by oversight bodies. To be able to do this, there must be a clear definition of the different categories and scales, so that input information is represented properly in the graphic model.

In addition to Bateson, several other authors have addressed the process of HBA in animal studies, providing the responsible entity with a broad range of helpful suggestions to consider in constructing their own systematic approach to HBA.

Checklists and key control questions

Some authors have also delineated useful checklists of keywords[7] or key questions[57] categorized under harms or benefits that responsible entities might find useful for inclusion in the development of an HBA. Authors generally have elaborated more expansive lists of harms than of benefits, and most authors have agreed that pain, injury or disease[2,7,30–32,37,38,40,41,49,60] and fear, anxiety or distress[2,7,16,26,30,31,37,38,40,41,49,57,60] are important harm factors (Table 2). The numbers of animals used, adverse alteration of the animals' environment and husbandry, impediments to normal behavior, duration of study, species and prospect of death are also frequently cited harm factors in the literature review (Table 3).

Process-oriented models

While the models above focus on different ways to categorize and present HBA, a process-oriented model, or sequential-question model[61] focuses on the process of how information is achieved and evaluated. This also includes the competence of persons involved in the process. In Figure 1 we describe a list of steps as an example that comprises a generic HBA.

Process-oriented models are generic and are structured around the HBA process. They safeguard how different opinions are represented and question the method and quality of the analysis. Process-oriented models do not provide answers on what model to use or provide solutions for conclusions.

Discussion

Classifying experiments in severity categories aids the identification of harmful experiments that require extra attention or resources. Such classification systems have been developed both in Europe[46] and in the USA.[48] Experiments causing severe harm warrant careful evaluation and the incorporation of applicable refinements such as the selection of alternative experimental parameters of satisfactory value, early intervention and other more humane endpoints. Such refinements can also be used as means to down-classify a specific experiment from a severe category to a moderate or mild category, with the overall goal of maximizing harm reduction in all cases as the objective in mind. Categorization also helps in the communication on how HBAs and evaluations are made in a transparent manner. In general most models depend on clearly defined categories for harm, referring to a severity

scale that enables a comparison of harms. Even if severity categories are well-defined, complexities remain in the estimation of harm levels.[60]

Responsible entities conducting HBAs should expect to encounter dilemmas on a regular basis. For example, the prospective analysis of many studies will involve the estimation of harm based upon similar cases or information from the literature or colleagues and such estimations may not predict outcomes sufficiently. Further, if a tendency towards utilization of a general or universal categorization system versus evaluating each particular case evolves over time, responsible entities conducting such reviews must remain attentive to the potential vagaries of each approved study/procedure to ensure prompt corrective actions if animal welfare is compromised. For example, a study involving a highly invasive procedure (surgery) categorized as severe and is performed by a highly proficient expert might be less harmful and fully permissible whereas a moderately severe procedure performed by someone less skilled may be perilous for the animal subject. Another example from the area of infectious disease would be the use of a standardized model of infection in which the dose of infectious agent, pathogenicity of the specific agent used, housing conditions and the associated treatments studied could have a profound influence on animal welfare. These distinctions deserve careful scrutiny in a thorough HBA. Also, they serve to re-emphasize that prospective HBAs should always be reconciled with an actual HBA performed retrospectively to help guide future decision-making by the responsible entities.

The species used has also been considered as a harm factor. Experiments using some animal species are regarded as more harmful than other species even within the same subphylum (vertebrates). There are some special restrictions for the use of non-human primates in Article 8 of the EU Directive.[2] Russell and Burch also stressed the importance of using less sentient animals as an alternative.[5] Extensive research studies have illustrated that phylogenetically lower animals have advanced social systems, communication and collaborating systems. Some regard fishes as less sentient animals and zebrafish have been used as an alternative model for mammals in studies of human disease.[62] However welfare states associated with the use of fishes are an emerging issue in international science programs.[63] Cephalopods are a group of animals that have recently been included and protected by the new EU Directive.[2] Research on these animals has discovered that they can not only experience pain, but they can also have cognitive abilities similar to what are recognized in advanced vertebrates.[53]

Harm is relevant for sentient animals or animals that can experience pain. Whether or not modifying an animal's genome is a harm factor depends on the ethical positions of those involved in the debate. Animal welfare advocates will only regard this as harmful if the modification results in a deleterious phenotype. A survey from Denmark showed that severe discomfort was only the case for about 15% of all genetically-modified animal strains, while 64% showed no discomfort.[64] The ability to suffer is a relevant harm factor. However, 'ability for suffering' requires consciousness in order to experience the harm. This has the implication that harm is only relevant for autonomously living animals and not a cell or an embryo.[39] Progress in gene technology has created a new ethical dilemma by facilitating the incorporation of new xenogeneic traits resulting in potentially deleterious phenotypes unknown in the natural history of the genetically-manipulated species. The level of consciousness of the genetically-manipulated species and the harm impact of the induced phenotype may warrant consideration in these cases. It is important to recognize that there might be conflict between a researcher's perception and the public's perception of what is ethically relevant.[39]

As harm is a result of planned activities, it can generally be estimated in advance, hence providing an intrinsic opportunity for the implementation of harm control measures (i.e. refinements). By contrast, benefit is more poorly defined; benefits have been categorized in domains, but not ranked in value comparable with the severity categories for harm. There is no clear hierarchy for benefits, with the exception that economic benefits or the benefits associated with improving vanity products would warrant less support than health benefits for severe diseases such as cancer or cardiovascular disease in humans.[10,65] Surveys indicate that the benefits of progress on less severe diseases or lifestyle diseases – such as obesity – are also less supported.[10] However, public opinion is often based on a simplified picture of the causality between a disease factor and disease, not reflecting the actual complexity behind a certain condition such as obesity, for example.[65] In such cases, researchers will be challenged and will have to explain very clearly what they want to achieve by using animals and why alternative approaches cannot provide equally valid information, while such a justification will be easier to sell in the question of a study of cancer, for example. Even if weighing benefits for different purposes against each other is difficult, Stafleu et al. have made an attempt to include benefit weighing in their model for HBA.[40]

Benefits of a particular project depend upon internal factors such as scientific and technical quality. Actual benefit also depends on external factors such as the usefulness of the data for immediate application or commercialization, or to future productive scientific endeavors ultimately deemed valuable by society. Although there are frequent instances where the

Table 5. Topics and discussion in ethical review of animal experiments.

Agreement with the following statements	Very much disagree or disagree	Neutral	Very much agree or agree
3R implementation is a fundamental ethical issue.	6%	5%	89%
Ethical committees, in my experience address all 3Rs in an equally balanced way.	53%	31%	16%
Ethical committees, in my experience, use most time discussing Replacement.	57%	33%	10%
Ethical committees, in my experience, use most time discussing Reduction.	21%	46%	33%
Ethical committees, in my experience, use most time discussing Refinement.	19%	0%	81%
The harm to the animals is a fundamental issue in ethical evaluation.	10%	0%	90%
The benefit for humans is a fundamental issue in ethical evaluation.	14%	14%	72%
The harm versus benefit is a fundamental issue in ethical evaluation.	10%	15%	75%
Scientific quality is an important part of ethical evaluation.	20%	15%	65%
Scientific issues are a major part of discussion in ethical evaluation.	29%	19%	52%
Philosophical issues are an important part of ethical evaluation.	60%	25%	15%
Technical issues are an important part of ethical evaluation.	11%	28%	61%
The right for humans to use animals in research, is a major part of discussion in ethical evaluation.	52%	10%	38%

Olson A, Kalman R and Brønstad A. Survey from FELASA workshop on ethical evaluation, Helsinki, Finland, June 2010.

researcher believes that a high value benefit is within reach, it is much more common for the beneficial value of the anticipated experimental outcome to be unknown. While harm is immediate, the certainty of a benefit can be unpredictable and intangible.[26,49]

In the EU Directive, potentially beneficial outcomes in research, education, testing and disease diagnosis may justify granting permission to use animals but only when there are no alternatives to animal use.[2] There is inherent uncertainty regarding the direct outcome and applicability of basic research. The investigation of basic mechanisms that are unknown or only partially characterized cannot be reliably conducted using alternative methods. While basic research is burdened with some uncertainty regarding direct benefits, we have a long history of experience showing that basic research is beneficial for the development of society, especially with regard to taking advantage of technological progress. For routine tests and educational activities, many alternatives are available and more appear to be evolving. Also, guidelines for the use of animals in educational applications have long contained the proscription that harm should not be inflicted solely for the purpose of educational or instructional activities. Learning outcomes and improvements in skill can be direct benefits of practicing procedures in a living animal. However, using animals for training purposes can be more easily replaced by alternatives than is the case with basic research.

There seems to be a stronger tradition of emphasizing harm, especially of harm reduction, than of considering benefits in responsible entities.[66,67] In a survey among workshop participants at a FELASA meeting (2010, Helsinki, Finland, unpublished; Table 5) 90% agreed or strongly agreed that 3R implementation is a fundamental ethical issue; however only 16% thought that the 3Rs are equally balanced. Most (81%) agreed that refinement is the R that got the most attention, and 90% agreed or strongly agreed that harm to the animals is a fundamental issue in ethical evaluations. Also the majority (71%) agreed that the benefit for humans is a fundamental issue in ethical evaluation and that harm versus benefit (75%) is

important in ethical evaluations. Only 38% reported that the right for humans to use animals in research is a major part of the discussion in ethical evaluations. In this audience, which presumably was more insightful and pragmatic than the general public on the matter of animal use in research, only 15% agreed that philosophical issues are an important part of ethical evaluations. Other studies have reported that discussions nearly unilaterally focus on details and technicalities more than on overall ethical judgments in the approval of animal research.[9]

The focus on harm in animal ethical review bodies might be because harm seems easier to assess for the reasons mentioned above. However, it might also be explained by the composition of the typical animal ethical review bodies having more competence and ease in the consideration of technical and harm issues. Evaluating benefits is more commonly performed by funding bodies which operate in a separate domain disconnected from the responsible entities. The split evaluation of harm versus benefit between these two separate authorities may alter the value and outcome of the HBA.[66,67]

The consideration of benefits in the HBA of animal studies shares some features with the consideration of benefits in the HBA of human clinical trials. However, in animal experiments, benefits seem most difficult to assess, while in human clinical studies harms are regarded as the more difficult part.[25] This might be because some uncertainty of risk for human participants has been eliminated in preclinical testing and the prospect of residual harm is scrutinized in detail, and because there is a chance that the participant will benefit from a new drug or treatment regimen.

Professional terminology for the discussion of harm is well established, i.e. 3R, severity classes, refinements, harm reduction, humane endpoints are all expressions used in the discussion of harm especially regarding harm minimization. A similar terminology for the discussion of benefits seems to be lacking; and benefits are discussed in more general terms, i.e. benefits for certain groups or purposes, but not in terms of actions to increase benefits. In our opinion, it would be in the interest of stakeholders who have a need to use animals to develop a wording to highlight the anticipated benefits for their animal experimentation.

Several models for categorizing harm–benefit and algorithms on how to balance these have been presented. We categorized the different models as algorithm models, graphic models (squares, cubes and decision trees), checklists and key questions and process-oriented models. All these models depend on some kind of categorization to simplify a complex picture into defined units that can be used as input information in the model of choice. Simplifications in categories and models to aid the HBA may detrimentally reduce: the quality of information, appreciation of the uniqueness of each proposal analyzed, moral sensitivity and attention of the responsible entities. Simplification may also favor routinization on the cost of cultivating an ethical vocabulary and creating distance to consequences of actions.[68]

HBA is dependent on the context, and context changes over time. Seventy-five years ago animals were used for pregnancy testing, such as the Friedman test in rabbits developed in 1939.[69] It was beneficial for women to know if they were pregnant or not. Today, in vitro kits for pregnancy testing are cheap and easily available commercially and women can carry out the test themselves. So even if it is still a benefit for women to know if they are pregnant or not, the use of animals for pregnancy testing is no longer morally acceptable because alternative methods have been developed. Decades ago central venous catheters were surgically placed routinely without imaging the final position of the catheter tip, causing complications from endocardial damage. Under what circumstances would conducting this procedure in this manner be considered acceptable today? Harm–benefit evaluations are dependent on the context and availability of alternative methods. Such alternative methods should be checked out at an early stage before large efforts are used in harm–benefit evaluations. Decisions in the past cannot take precedence over decisions in the future because technological solutions as well as research questions that need to be answered will change as research and technological developments advance. For ethical evaluation of an animal experiment, the context in which the animal experiment is done will always be relevant.

Some AEC members expressed that they have always been doing harm–benefit evaluations while others expressed concerns that doing HBA is something new and that it changes the role of the AECs.[67] Though models to illustrate the harm–benefit concept like the Bateson cube[16,37,52] are known, such models are not operational as long as they lack clear explanations on what to put in the different boxes in the model. Algorithm models[32,40] are more instructive but are criticized for reducing moral questions to arithmetics.

Although harms and benefits have been systemized and categorized, and there may be common understanding and agreement on these categories, weighing harms and benefits will be biased depending on the individual persons responsible for the discussion.[27] Attitudes to animal experiments influence decisions of being in favor or disfavor of particular animal experiments.[10] Savadori et al. have demonstrated that such biases, also called affective heuristics, do not only influence the decisions of lay people.[14] Affective heuristics also influence opinions of experts, however to a lesser

degree than to lay people.[14] Based on this fact it might be reasonable to suggest that different interests and/or competencies should be present in the discussion of HBA to enlighten different perspectives and views of a particular case. A broad representation of interest minimizes some – but never all – biases caused by affective heuristics.

A reason why people find harm–benefit evaluation difficult is that it actually challenges personal attitudes to animal experiments, to animals as sentient beings and to the relationship between animals and humans. Anyone who is part of such decision-making is responsible for this, and this is different from ticking checkboxes, checking compliance with regulations, guidelines or the 3R principles.[66,67] Compliance with regulations is required but does not encompass the totality of ethical behavior. Ethical responsibility is a domain placed higher in the hierarchy than legal responsibility.[70] Sometimes regulations have limitations in providing a good solution and there is a need for making a 'wise' decision for a particular case. This is an important decision for the responsible entities.

The working group defines HBA as a transparent systematic method to gain information about harm to animals and expected benefit so that qualified decision of approval or rejection of projects can be made.

Ethical 'tools' are different 'rules' used to justify or solve moral dilemmas. An example of such a tool is the 3Rs.[5] These are intended to minimize harm to animals where possible by using alternative biological systems or replacement methods; to reduce the numbers of animals used through animal model selection and in vivo methods that improve data compactness; or procedural refinements to reduce suffering. No 'tool' or model is perfect, and though the 3Rs have gained support by moving animal experiments in a better direction there are still several conflicts hidden within the 3R principles.[36] Furthermore, unilateral focus on the 3Rs draws attention towards harms and the negative aspects of animal experiments, while not taking the potential positive outcome (benefits) or context into consideration.

HBA serves as a foundation or comprehensive ethical maxim, which subsumes later application of ethical tools such as the 3Rs in the construction of humane animal experiments. To justify studies that are anticipated to inflict harm to animals, there must be a reasonable expectation of specific benefits commensurate with the level of harm; or there must be an urgent scientific need with profound implications, despite the suffering caused, that cannot be fulfilled using other methods.

However, harm and benefits are not necessarily the only considerations made in the final decision. Kvalnes and Øverenget proposed the'navigation wheel' as a model for decision-making to keep track of relevant decision-making factors that also include factors other than ethics in decision-making.[71]

The utilitarian concept of justifying the use of animals in research has existed for decades and also seems to be applied independently of attitudes to animal experiments.[10] Utilitarianism or consequentialism is an ethical philosophy holding that the proper course of an action is the one that maximizes utility (i.e. maximizing happiness and/or reducing suffering).[8,72,73] Jeremy Bentham and John Stuart Mill were important contributors to classic utilitarianism. Singer, an Australian moral philosopher specialist in applied ethics and author of the book *Animal Liberation*,[34] also holds a preference for the utilitarian perspective. According to Singer the interests of animals should be considered because of their ability to suffer, and he argues that animals should have rights based more on their ability to feel pain than on their intelligence. Ability to suffer was also the main relevant property according to Jeremy Bentham (1748–1832):

'*The question is not, Can they reason? nor Can they talk? But can they suffer?*',

Ethicists may disagree that HBA is uniquely utilitarian as utilitarian ethics is often based on the assumption that the welfare of animals and humans is equally important. However, when performing an HBA in the context of animal experiments, human health and welfare interests usually count for more than animal health and welfare interests. Though HBA is based on maximizing utility, it also has elements of contractarianism, an ethical position placing human or one's own interests first.[74]

One of the success factors of HBA is that it makes sense from different ethical perspective. However this can also be a source of conflict as different people, with different stances, are influenced by affective heuristics[27]

Box 5. Ethical stances explaining the success of HBA.

Peter Sandøe (personal communication)

1. A utilitarian perspective where equal needs should be treated equally, irrespective of whether these needs are human or animal – so harms to animals are only allowed if they are justified by a higher likely benefit (and if there is no alternative line of action with a better harm–benefit balance).
2. A contractarian perspective where HBA is all about ensuring minimizing animal harm and maximizing human benefit, without questioning the basic speciesist premise that animals are for us to use, and without really trying to compare the size of the animal harm to the likely human benefit.

and therefore have different expectation as to what HBA can offer, and this thereby gives rise to new controversies rather than solutions.

An important side-effect of performing a thorough HBA is that the responsible entity and the scientist work carefully to incorporate the 3Rs to the fullest degree compatible with the scientific question being posed. This collaborative commitment to the 3Rs likely helps foster a positive public perception of animal use in research.

Regulatory compliance provides a license to operate. Regulations are commonly based on past knowledge, and may therefore be out of date. While regulatory compliance is demanded, and is important, it may only contribute minimally to the formulation of broader ethical decisions. We expect that doing harm–benefit assessment will stimulate scientists to investigate alternative experimental approaches in light of the current context. In our opinion HBA stimulates ethical reflection, dialogue and discussion on the use of animals in research. Assessment of harms and benefits enables researchers, reviewers and funders to decide whether a particular experiment is worth doing at all.[26]

Conclusion

The AALAS–FELASA WG on HBA has reviewed the existing literature on this topic and defined the current concept and elements of HBAs. Harm is a negative impact on the sentient being. There is consensus that sentience and ability to suffer are relevant. There is also agreement that harm is more than pain and suffering, and includes all sources that can cause negative impact on animals, and harms can be related to all domains provided by the five freedoms. Disrespect for life is a harm factor. Some authors also discuss genetic manipulation as an infringement in itself, while others regard this as harmful only as long as the genetic modification causes an impaired phenotype. Designing experiments performed with total anesthesia where the animal will not regain consciousness is regarded as a refinement and a way to limit harm experiences.

Benefits for human, animal or environment health are regarded as acceptable benefits to justify animal use as long as there are no alternatives. The intrinsic uncertainty whether promised benefits will be realized or not, must be compensated by strengthening the quality of the study to optimize the possibility of reliable known benefits.

The quest for knowledge, safety and forensic purposes are also regarded as legitimate justification. Economic interests or benefits related to vanity products are less acceptable benefits; however such studies may also have other favorable secondary benefits. Primary or actual benefits seem easier to accept than secondary benefits because the relation between the experiment and outcome seems more likely.

Several models for comparing and weighing harm–benefits are presented. The Bateson square and cube have gained recognition as models to illustrate the concept of HBA. This is a good model for illustrating the concept, but is less operational as long as there are no clear rules for what to put in different cells in the cube. Algorithm models have also been presented but are criticized for reducing ethical issues to arithmetic exercises. Checklists and key questions are useful for checking that important relevant harm and benefit factors are addressed. Subjective opinions will influence decisions in a committee responsible for HBA. A broad representation of different legitimate interests in the decision-making group is therefore recommended, as provided in a process-oriented model. Whatever model is chosen it should be transparent so that it is possible to verify what harm and benefits were evaluated and on how much weight is put on each of them. Finally, the best solution may be found by combining aspects from the different models.

The working group defines HBA as a systematic, transparent way to assess and compare harms, benefits and how they are balanced.

HBA is valuable in that it stimulates ethical discussion and reflection; it questions current practices and is therefore a driving force for improvement and ethical decisions. HBA identifies harm and drives researchers to seek alternative approaches to reduce or eliminate harm (refinement), and is important in avoiding uncritical use of animals just for the cause of the good. HBA is based on the assumptions of maximizing utility for the majority where human interests count most and is an essential part of the ethical review. Since HBA drives ethical reflection and discussion on current practices, it is important for building public support to ensure that harm to animals is taken into consideration and that animals are only used to achieve legitimate important benefits. Decisions based on a particular HBA are dependent on and limited to the current context.

In Part 2 of the WG report a method for analyzing harm/benefit is provided.

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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]

Abstract

International regulations and guidelines strongly suggest that the use of animal models in scientific research should be initiated only after the authority responsible for the review of animal studies has concluded a well-thought-out harm–benefit analysis (HBA) and deemed the project to be appropriate. The AALAS–FELASA working group on HBA has performed a literature review and based on this review, proposed a method for HBA. Examples of the working group's approach are included in this report.

Keywords

harm–benefit, ethical review

Background

International, regional and national guidelines provided by the Office International des Epizooties (OIE),[1] Council for International Organizations of Medical Sciences–International Council for Laboratory Animal Science (CIOMS–ICLAS),[2] the European Directive,[3] European Science Foundation[4] and the *US National Research Council Guide for the Care and Use of Laboratory Animals*, 8th edition (*NRC Guide*)[5] offer impetus for responsible entities to pursue harm–benefit analysis (HBA) during the ethical review process of animal experiments. Of note, none of these guidelines offers any parameters for what constitutes an appropriately rigorous HBA process.

The American Association for Laboratory Animal Science–Federation of European Laboratory Animal Science Associations (AALAS–FELASA) working group (WG) on harm-benefit analysis has defined HBA as a systematic process for assessing and comparing the harms and anticipated benefits of a particular animal study.[6] The establishment of a systematic process for HBA is expected to ensure that all potential harms and benefits have been comprehensively and carefully considered during the ethical evaluation of the merits of an animal research investigation.[7] This approach entails evaluating each component or procedure of a project for harm and considering the relative importance and relevance of the evidence (benefit) it potentially contributes to the hypothesis being tested.

A systematic HBA should help optimize the protection of animals from all undue and avoidable harms, improve consistency, completeness and transparency of the ethical evaluation, and result in a sound ethical justification for studies deemed to be scientifically valuable. The HBA helps formalize and structure the

[1]Chief, Comparative Medicine Branch, NIEHS/NIH, Research Triangle Park, NC, USA
[2]AAALAC International, Frederick, MD, USA
[3]Chief Veterinary Officer, Sanofi, Marcy l'Etoile, France
[4]Department of Laboratory Animal Science, GlaxoSmithKline, Research Triangle, Park, NC, USA
[5]AAALAC International, Pamplona, Spain
[6]University of Bergen, Department of Clinical Medicine, Bergen, Norway

Corresponding author:
Kathy Laber, National Institutes of Environmental Health and Sciences, 111 TW Alexander Drive, D108, Building 101, Research Triangle Park, NC 27709, USA.
Email: kathy.laber@nih.gov

information needed to make an informed consensus decision on whether the benefits of performing an experiment outweigh the potential harms posed to the animals used in research and subsequently, whether the proposal should be accepted or rejected.

A review of the literature shows that several methods of HBA have been described, and current concepts of HBA are summarized in the *AALAS–FELASA working group report on harm–benefit analysis – Part 1*.[6] Recommendations on how HBA can be addressed and implemented by responsible entities as part of the ethical evaluations of protocol/project applications was the other task assigned to the AALAS–FELASA WG, and is the focus of this report.

Introduction

Persons responsible for the protocol/project applications must ensure that animal welfare is considered comprehensively according to current concepts of harm[6,8,9] and also that harm is mitigated, for example by implementing 3R (replacement, reduction and refinement) actions.[10] Harm to animals is a public concern and it is not limited to pain alone. With regard to benefits, researchers must explain in plain language what the expected benefits are and they must also explain why certain harms might be necessary to achieve those benefits. Furthermore the information relevant for HBA must be presented in a way such that reviewers can see what harm and benefit factors have been evaluated as well as see how they have been considered. This is important for transparency of the process and to clearly understand how the decision on approving or rejecting a particular project was evaluated by animal ethical committee (AEC) members.

The AALAS–FELASA WG on HBA suggested framework and approach for HBA

The WG recommends a systematic approach to HBA by using a template to address all relevant aspects of harm and benefit. A template will have a normative impact; the researcher will know what harm factors are relevant for consideration which should help to promote refinement[10] and, similarly, will know what expected benefits are anticipated that are in accordance with regulatory guidelines as well as in line with public perceptions on acceptable uses of animals in research. Also, standardization of the assessment approach is one way of increasing consistency in ethical assessment.[7]

In the following we describe a method of HBA using such a template. Based on literature reviews and discussions of the pros and cons with different models,[6] we synthesized a new model for HBA utilizing components from previously published models. This approach entails the broad consideration of harms based upon the five freedoms[9,11] and affords the consideration of a diverse spectrum of benefits. This tool should permit responsible entities to extract relevant information from research animal proposals in support of a deliberative and transparent HBA. Examples on how to evaluate harm and benefits are provided in the discussion. Examples of two mock research proposals are presented in Appendix 1 (Examples 1 and 2), and Tables 1 and 2 in Appendix 1 provide examples of how to use the model/tool presented by the Working Group.

Framework for evaluation of harm/benefit

First, the animal proposal that is used by committees to evaluate harm/benefit should be framed in a manner that illustrates why a particular study that uses animals can be expected to be of value, and should contain the details needed to allow the reviewers to determine the harms.

To aid in defining the harms, the WG chose, as did Mellor,[8,9] to consider harm factors that compromise animal subjects within the categories of the five freedoms[11] (see Key in Table 1). This approach offers a comprehensive and broader view on animal harm and welfare, which we believe better safeguards the interests of the experimental animals[8] and identifies important areas for the application of the 3Rs.[10] The five freedoms[9,11] are used to define the overarching harms of the study.

The benefits for the study are defined using an overarching set of domains that was derived from the literature review (see Key in Table 1). It is clear that benefits from applied, immediately translational research are easier to define than possible benefits from basic research, but the importance of the benefit is not correlated to the ease of its definition.

The WG acknowledged that ethical review committees are presently well positioned to assess 'harms' but may be less well equipped to conduct a benefit analysis.[12–14] It may then be necessary to incorporate work from other review bodies such as scientific granting agencies and scientific peer/specialist review committees. However the summation of both 'harm' (Table 2a) and 'benefit' (Table 2b) tables needs to be linked in order to conduct a 'harm–benefit' analysis.

The following steps define the process for HBA:

1. Detail the harms and benefits at the top of both the harm (Table 2a) and benefit (Table 2b) tables.
2. Engage in a systematic review of how different animal, experimental, and environmental variables affect, or modulate, the harms associated with the

Table 1. KEY: Benefit Domains and Harm Factors.

Benefit Domains
Social benefits • Human health • Animal health • Environmental health
Socioeconomic benefits
Scientific benefits
Educational benefits
Safety and efficacy

Harm Impact on Five Freedoms
Freedom from Pain/Injury
Freedom from Fear/Distress
Freedom from Hunger/Thirst
Ability to express normal behavior
Freedom from Discomfort/appropriate husbandry

proposed animal-based experiment. A suggested list (and definitions) of 'modulating factors' (MFs) for harms are identified and listed in the relevant table; however, this list can and should be adapted as needed based on project or institutional circumstances. Individual harm MFs are frequently interrelated and may overlap.

3. Once the list of MFs for harms is defined (Table 2a, column one), a brief description summarizing the critical point for analysis of each of the MFs in the context of the project is included (Table 2a, column two). For example, the housing conditions of the animals used in the project can be described, and details of the type and size of caging, and social/individual housing conditions can be included.
4. Depending on how the MF is applied in the context of the project, it may mitigate and/or aggravate the harm inflicted on the animals. While in some instances the effect may be only aggravating or mitigating, in others both effects may exist and should be considered. For example, if, under the 'housing' MF, the study requires that social animals be individually housed for a period of time, this would be interpreted as an aggravating factor, but if they are also provided with a very good enrichment program, with access to open areas and human contact, there would also be a mitigating effect, which would balance the final outcome for this particular MF. These descriptions should be included in the 'mitigating effect' and 'aggravating effect' columns.
5. The summary of the mitigating and/or aggravating components of each MF is depicted by a summary color or score (see Table 3). The color gradient scheme facilitates an easy and intuitive interpretation for the outcome of the MF analysis. We decided to use grades of red, indicating a heat map for the HBA: the deeper red, the 'hotter' the HBA is towards rejection of proposal. 'Cold' or white experiments or those with a hint of pink are easier to support. Numbers have intentionally not been used, to avoid the temptation of letting 'calculation' guide the decisions.[26] Traffic light colors could also been used as suggested in a modified Bateson model.[15] However we think that the green color used for acceptable experiments (low harm–high benefit) gives a false-positive impression that animal experiments are acceptable, while we think that animal experiments always raise ethical concerns, and there are just shades of acceptability based on the harm–benefit balance.

As an example, if 'species' is used as an MF, crimson could be assigned to the use of non-human primates if a lower phylogenetic species could be substituted. Similarly for the 'housing condition' MF, social housing of dogs in pens with access to outdoor areas and a very good enrichment program could be assigned a white color, compared with the use of a crimson color for individual housing in small metabolic cages.

If the details of the MF result in a dominant mitigating effect, the final color assigned in the 'summary color' column would be white, or '–' if scoring is used, and a clear aggravating effect would have a score of '+++++' and a low aggravating effect would have a score of '+'. Note, the 'scoring

Table 2a. Harm table.

HARM TABLE

HARM-Freedoms Impacted
Pain/Injury: _____
Fear/Distress: _____
Hunger/Thirst: _____
Ability to express normal behavior: _____
Discomfort/Husbandry: _____

Modulating Factors for HARM	Description	Mitigating Effect	Aggravating Effect	Summary Color
Animal— Species				
Animal— Number				
Animal— Suited to environment				
Animal— Health status				
Experimental- Intensity				
Experimental Duration				
Experimental Cumulative Experience				
Experimental—Endpoint				
Experimental Complication/Distribution Rate				
Experimental Genetic Modulation				
Environmental Housing/Husbandry				
Environmental Personnel competence/experience				

system' gives each category a discreet quantitative value that may give the misleading impression of a precise arithmetic assessment, whereas colors provide a wider spectrum that allows for a more intuitive and visual result. This is particularly important in view of the variety of MFs and the different weights that each may carry (protocol-dependent).

6. As with the harm table, the MFs for the benefit domains are defined and listed (Table 2b, first column). The MFs for benefits should help elucidate

Table 2b. Benefit table.

BENEFIT TABLE

Social
- Human health: _____
- Animal health: _____
- Environment health: _____

Socioeconomic: _____
Scientific: _____
Educational: _____
Safety and Efficacy: _____

Modulating Factors for Benefit	Description Why/How/What /When	Summary Color/ Numeric Score
Importance of outcome		
Clarity of objectives		
Translational Potential		
Likelihood of success		
Continuity of recognized scientific efforts		
Quality of Experimental Design		
Innovation Level		
Dissemination of Results		

to the user the 'what, why, how and when' the benefit will be realized.[15] The WG concludes that a summary color could be assigned for each MF but that individual mitigating and aggravating circumstances do not apply.

7. Once the harm/benefit tables (Tables 2a and b) have been completed, committees can visualize, from either the color or scoring system, the overarching intensity of the harm and the expected strength of the benefit, and make decisions on whether the proposal should be approved, rejected or modified. As an example, if the harm is intense, and the benefit minimal, the committee should reject the proposal, or work to implement approaches such as reduction, replacement and refinement, that would lower the harm level. If the benefit is high and the harm low the committee could, without reservations, ethically justify approval of the proposal.

In the process of evaluating all potential harms and benefits in a systematic fashion, it is essential to realize that the weight or significance given to an individual harm/benefit will not be equal, and a single harm or benefit factor could dominate and steer the final outcome.

Table 3. Summary of color gradient and score scheme for harm/benefit factors.

*Category:	Category Description:	Color Score:	Plus Score:
Harm/Aggravating Factors	No impact	White	-
Benefit /Strength Factors	High Impact		++++
Harm/Aggravating Factors	Minimal	Pink	+
Benefit /Strength Factors	Moderate		+++
Harm/Aggravating Factors	Mild	Rose	++
Benefit /Strength Factors	Neutral		++
Harm/Aggravating Factors	Moderate to Severe	Red	+++
Benefit /Strength Factors	Minimal		+
Harm/Aggravating Factors	Severe	Crimson	++++
Benefit /Strength Factors	No positive impact		-

Definition of MFs for harms

Animal.

Species: Species proposed for the project. Potential relevant factors include: sentience, cognitive ability, phylogenetic scale, adaptation to laboratory conditions, rarity and societal concern.

Number: Total number of animals (by species) to be used in the project.

Suited to environment: Origin (source) of animals and acclimatization procedure.

Health status: Clinical/subclinical condition, which could cause harm to animals. Experimental and spontaneous genetic mutants that have adverse phenotypes should be considered.

Experimental.

Intensity of harm: Descriptions of experimental procedures that compromise the five freedoms, and measures to alleviate them.

Duration of harm: Description of the immediate impact on the five freedoms and measures to alleviate them, e.g. temporary single housing of social animals.

Cumulative experience: Total periods of time over the animal lifespan where the five freedoms are impacted (e.g. animals that are reused for pharmacokinetic studies over their entire lifetime).

Endpoint: Explanations on how/if endpoints ensure animals are not subjected to unnecessary suffering, i.e. refinement, including observation procedures.

Complication/distribution rate: Distribution of the impact of harm among study animals and/or relative proportion of study animals subjected to different severity levels. For example, the total number of animals in a study is 100, but only 10 will be subjected to severe procedures.

Phenotypic manipulation: Genetic, surgical and chemical modifications that result in impact on animal well-being as part of the experimental model.

Environmental.

Housing conditions: Enclosure sizes and characteristics; social–individual housing; environmental enrichment.

Husbandry: Quality and provision of food, water, sanitation and identification.

Personnel: Competence of animal care personnel with regard to the care of the study animals, and competence of the research team with regard to the experimental procedures.

Definition of MFs for benefits

Purported importance of outcome: 'WHY' *is the study important*? Although this cannot be defined with certainty an estimation of the importance of the outcome

of the study should be made. This can be framed in terms of immediate and short-term benefits as well as the anticipated impact of the outcome for subsequent studies and long-term benefits.

Clarity of objectives: 'HOW' *will the objectives be met*? and 'WHAT' *will the objectives be*? The degree to which a sound hypothesis and clear objectives are elucidated can support the driving purpose of the study and ensure that the study outcome has value/benefit.

Translational potential: 'WHO' *will the study benefit*? and 'WHEN' *will the benefit be realized*? An assessment of how feasible the study is and how quickly the results can be expected to be applied to the benefit domain.

Likelihood of success: 'HOW' *likely is it that you will obtain the objectives desired*? In addition to the complexity and difficulty of proposed studies there are several other factors that affect the likelihood of success. These factors may include the existence of appropriate facilities, the expertise and competence of research and animal care and use personnel, as well as the level of resources and funding available to assure completion and continuity of the work. The track record of the study team should be considered in this evaluation.

Continuity of recognized scientific efforts: 'WHAT' *is the larger body of knowledge this study contributes to*? Consideration of how well this work amplifies/adds to the continuum of knowledge gained from previous studies, or indicates whether there is potential to continue to offer further benefits.

Quality of experimental design: 'HOW' *will the objectives be obtained with high quality/effective use of resources (animals, time, etc.)*? The quality of the experimental design should be considered in the benefit equation in that it ensures that the data collected are scientifically acceptable, and will validate the results obtained.

Innovation level: 'HOW' *will this study advance science beyond the specific objectives of the study itself*? Consideration of whether or not the proposed research will benefit other research through the conduct of novel and innovative processes and designs. This may include expected secondary benefits such as 3R advances.

Dissemination of results: 'WHEN' and 'HOW' *will the results be distributed*? How will the results be disseminated for maximum benefit? (e.g. are the results proprietary or public; presented or published, etc.).

Discussion

Are all harms equal?

Drawing on information from the literature[6] and the WG professional experience, the WG offers the following points for consideration in the systematic analysis of harm.

There are inherent challenges in assessing procedural severity, and the application of professional judgment is often warranted. The level of harm is influenced by the quality of facilities, equipment, housing conditions (social versus single, quality of environmental enrichment, etc.), staff and investigator skills and competence, quality of veterinary observation and care, individual species and animal issues (phenotype and health status) as well as the definition and implementation of experimental endpoints. In summary, the level of harm is not related exclusively to the nature of the experimental procedure, but also to many other variables. Responsible entities should evaluate these elements carefully during HBA to ensure that high competence and appropriate compensatory provisions for procedures potentially impacting animal welfare and harm are applied in every instance to the fullest extent possible.

Example: Conducting procedures in a specialized center for studies with a particular species with unique requirements may result in far less stress to the animals involved than to animals used in similar studies conducted in research facility environments without similar personnel, knowledge, expertise and equipment resources suited to the species and research investigation. Responsible authorities should ensure that essential resources and expertise are in place before allowing studies to proceed.

The species and the behavior of the individual animal are potentially important factors when determining the level of harm. The same procedure may be scored differently depending on the species or the native reactivity and prior acclimatization. For example, a procedure conducted in a species that typically tolerates it poorly will normally be considered as more harmful than if conducted in a species that tolerates it well. Also, within a species, some individuals may be better acclimatized to experimental conditions or may have a naturally more cooperative disposition than others in behavioral studies.

Example: Comparable stereotaxic surgical procedures in neuroscience studies can be performed in non-human primates and in rats. Although in both cases the pain associated with the procedure itself can be abolished by means of appropriate anesthesia and analgesia, the level of stress/distress created by captivity may differ by species. In addition, differences in research environment, competency of personnel (see previous examples), housing conditions, etc. may affect the final level of harm associated with the procedure.

Consideration of harm should take into account the cumulative experience of the research animal in the research facility as well as in experimental procedures.

Example: Responsible entities may wish to develop specific guidance concerning repetitive procedures they will allow in animals that are maintained for specific purposes. For instance in colonies of animals instrumented for safety pharmacology studies significant factors that

impact harm include: how long would animals be maintained, how many drugs would they be exposed to, and how long would they be 'rested' between procedures. The harm to animals would not be solely defined by the experiment proposed but also by the background of the use of the research subject.

Harm analysis should balance individual animal needs with those of the entire experimental group cohort used for data acquisition. Responsible entities should be receptive to assessing whether procedures of a greater severity to a few individuals are warranted instead of conducting procedures of less severity to a greater number of individuals in certain circumstances.

Example: Maintenance of a small colony of cannulated animals for repeated metabolism studies subjects a small number of animals to surgical procedures and repeated doses of compounds and procedures to which they become well adapted. This might result in less cumulative harm than would occur if multiple animals were used in single experiments for which the individual bleeding procedures were more stressful and for which they were not as well adapted.

For all animals, including genetically-modified animals, harm should be assessed using observation and scientific measures of pain and distress that occur in the research subjects and not by the assumption that alterations from the natural or wild state are deleterious a priori. Genetic modification does not necessarily result in experienced harm per se.

Harm should be assigned at the level of the whole research proposal submitted to the oversight body and should encompass all experimental procedures/conditions that potentially impact animal well-being.

There should be a mechanism of evaluating the actual level of harm regularly during the development of the protocol/project. If the harm seen is different from the prospective harm assessment, responsible entities should re-evaluate and take appropriate action. Moreover, responsible entities cannot reliably be expected to achieve a sound review process if the assessment is entirely conceptual and is limited to just a paper review.

Pilot studies are useful for determining the in vivo experimental approach and types of procedures to optimize data collection. Sometimes, however, very little prior knowledge is available to predict expected outcomes and the harm experienced by the animals. Thus, special attention in the evaluation and the actual conduct of pilot studies should be provided.

Humane endpoints can reduce the level of pain and negative impacts on the five freedoms. In some cases, researchers need some preliminary data to determine effective, early endpoints. The capacity to define early endpoints clearly impact the HBA.

Are all benefits equal?

At a cursory glance, benefits of improving health caused by serious diseases are easy to support.[16] However, the nature of the underlying cause of a specific disease can be an issue for discussion.[16] Is the disease caused by predetermined factors (e.g. genetics), factors out of the individual's control (e.g. contagious disease, intrauterine environment or exposure, or accident), or is it a consequence of a certain lifestyle (e.g. smoking) where one might expect the patient to have some influence on the outcome, or is it a result of another deterministic factor? For many types of disease there are no discrete and identifiable influences but rather undefined and complex mechanisms that are at play, and caution must be taken not to categorically devalue benefits for diseases that have a 'lifestyle related' ethology.[17] Animal experiments are usually used as one tool, together with in vitro methods, epidemiological studies, clinical research or other scientific approaches. Therefore, in such cases the HBA should consider the use of animals as an additional factor in the approaches used to improve health.

Questions can be raised regarding routine product testing. Is the product of substantial importance for the improvement of the consumer's quality of life or is the product being developed to satisfy human pursuit for luxury items or for vanity reasons? This distinction is not always clear. Product testing clearly is designed to protect health, and some products are used for several purposes, for example the botulinum toxin is used both for treating wrinkles as well as for treating neurological diseases. The European Commission has already limited animal use in favor of alternatives in some circumstances through the registration, evaluation, authorization and restriction of chemicals (REACH) legislation[18] Also, placing restrictions on the commercial or intellectual freedoms involved in pursuing new drugs to improve performance and reduce side-effects is difficult, and few would propose defining a minimal increment of improvement necessary to justify the use of research animals in drug development. Responsible entities will undoubtedly be faced with assessing difficult ethical quandaries akin to the above on an individualized basis.

Scientific discovery efforts are often met with failure and the communication of these failures should be encouraged and facilitated and reported to ensure that negative findings bear some benefit. The publication of negative results may be regarded as counterproductive and a waste of time, potentially stigmatizing the laboratory and the research sponsor, and drawing all possible sources of failure of the laboratory into question. However, negative results are highly relevant because they reveal important knowledge and may prevent subsequent unproductive or poorly conceived

inquiries in the same area, subjecting additional animals to futile experimentation. According to Claude Bernard, the founder of modern medicine, 'there are no unsuccessful experiments . . . the results are always the true consequence of the conditions of the experiment'.[19]

The likelihood of success is a relevant dimension in the discussion of benefits. This does not relate to the uncertainty implicit in basic research, but to what extent the experiments are based on good scientific principles, a clear hypothesis and problem formulation, systematic review of existing knowledge, selection of appropriate methods, and research design to generate reliable data. Also, the likelihood of success depends on the research group's expertise and available resources (knowledge, skills, personnel facilities, etc.). Likelihood of success or quality of the experiments using the chosen methods and models was presented as a separate dimension from harm and benefit in the Bateson cube model.[20,21] We found it appropriate to discuss this under the likelihood of achieving the desired benefits and as an MF among the benefits, but did not give it its own dimension. Failure in design can lead to an unnecessary use of animals, publication of invalid results, and subsequent experiments being based upon flawed hypotheses.

There are enduring approaches to the systematic analysis of scientific problems and their investigation. The analysis logically begins with the systematic review of scientific literature relevant to the problem of interest.[22–24] Such literature reviews should be structured, thorough and transparent. Once the problem in question and experimental hypothesis are clearly formulated against the backdrop of a thorough literature review, the strategic selection of methods may proceed. This is important because any harm to animals can only be justified if it is really necessary to answer the question. This approach to the conduct of research was emphasized by Bernard[25] who stated, 'Like investigations in any field of science, the merit of animal experiments ultimately depends on rigid adherence to principles of the scientific method.'

Animal research projects funded through public resources and foundations are usually subjected to the critical, peer review of the research by experts in the field who declare their independence and absence of any conflict of interest in the conduct of their duties. The WG believes that peer review of this nature may constitute a factor important to a project's benefits and may serve as the nucleus of the responsible entities' final evaluation of benefit in the HBA process.

Simplied HBA

HBA using the approach described here can be a time and labor-intensive task. Therefore, responsible entities may wish to prioritize experiments according to the intensity of review and analysis deemed appropriate. A simplified Bateson square[20,21] can be used to devise a 'quick and simple' way to sort experiments. Animal experiments can be simply categorized as 'low harm–high benefit', 'low benefit–high harm', 'low harm–low benefit' or 'high harm–high benefit'. The low harm–high benefit experiments elicit minimal controversy. Terminal procedures (animals anesthetized for the whole experiment and then killed under anesthesia) for a beneficial purpose experience minimal harm, assuming that high standards of care are addressed. However, sacrificing a large number of animals in an experiment of this type would still raise ethical concerns if adequate scientific justification for high animal numbers were lacking. Experiments categorized as low benefit–high harm might also be easily decided. Very likely such experiments would not be ethically justified and the application would be rejected. Review of such a research proposal should prompt greater clarity on the nature and urgency of the expected benefits and should focus on reducing harm (refinement/3R). Experiments defined as low or moderate harm–low benefit or high harm–high benefit might stimulate the most discussion. Should the responsible entity accept the justification for an experiment where the benefit is poorly defined, even if harm for the animal is trivial? Experiments that are obviously beneficial but that also cause much harm are also difficult to assess. If newly emerging severe diseases occur, this might cause an urgent specific research need. Because experience with a new disease is limited it may involve some trial and failure before a research model is adequately refined to reduce harm. Finally, perhaps a majority of animal experiments (and animals) fall into a gray zone of uncertain potential benefit and experience harm levels that are not severe. In some instances, it may be permissible for responsible entities to reduce or waive the HBA review requirements in studies of this nature.

Responsibility for HBA Outcome

The model suggested in this presentation does not say anything about who should take part in the HBA and make the final decision. However, as subjective opinions influence our evaluations[27–30] we think a broad representation of competent persons is the only way to give a balanced HBA process and decision. This applies both for the harms and the benefits.

The WG favors and recommends the use of consensus rather than voting for the decision method of the responsible entities in conducting HBAs. The responsible entities should be able to project transparency in how they evaluated harm and benefit and how they

reached their final conclusion. This transparency will aid external review by outside groups if warranted, improve information exchange, and build broader, more informed consensus and aid communication on why animals are used to the general public.

Reaching an agreement about the relevant parameters of harm and methods to palliate them is far less challenging than reaching an agreement on what constitutes a meaningful benefit resulting from a research animal study. This is clearly evident in the chart summarizing the consideration of harms and benefits in the literature for research animal studies in which the two most cited categories, benefits to humans and the quality of the research, are identified as pertinent.[3,21,26,31] Identified benefits to animals, benefits to the environment, and knowledge benefits are also important considerations.[3,26]

The WG contends that the researchers must be able to define and describe some primary benefits for their inquiry while recognizing that there is no guarantee that projected benefits will be realized. Communications from both scientists and members of responsible entities with the WG have emphasized that the definition and assessment of benefits, particularly tangible benefits, can be a daunting and unsatisfying task in some studies. They have argued that for scientific projects that have undergone authoritative, external scientific peer review successfully and been deemed worthy of support with public funding, this should constitute an adequate, if not definitive, statement of the project's benefits. This approach should help address the growing administrative burden that scientists face from the responsible entity overseeing research and expedite decision-making. However, unless harm to animals has also been carefully addressed in the peer review process evaluating benefits, the conclusions of the external peer review may be brought into question and the ethical implications of animal use for the specific project has not been addressed.

Finally it is important to recognize that the final comparison and evaluation of HBA will be influenced by both attitudes and competence of those making the decision.[32]

Conclusion

The AALAS–FELASA WG on HBA has presented a model for conducting a broad, inclusive and transparent HBA. Impact on the five freedoms has been used to assess harm as this approach incorporates most of the harm parameters identified in the research animal literature and should serve other responsible entities in the thorough evaluation of harm. The central benefits encompass the advancement of human and animal health, knowledge and safety protection for humans, animals or environment. We recommend using standard qualifying questions like 'who, what, when and how' to help define how benefits will be realized.

Although there are ways to grade harm and benefit presented both here and by others, there is no common 'currency' or value system for comparing the different realms of harm and benefit. Therefore, HBA remains intractably context-dependent. The complex moral issues inherent in some HBAs are resistant to convenient automated decision making by use of algorithms and decisions will depend on individuals' moral consciences and value judgments concerning harms and benefits for a particular project. When implementing HBA the responsible entities should be represented by different stakeholders to give a balanced evaluation and a group consensus should be the desired outcome.

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Appendix 1: Examples of applying the tool

Protocol Example 1: Influenza infection and treatment with new chemical entities in mice

Description

Influenza virus (IFV) infection continues to be a significant unmet medical problem, requiring hospitalization of infants; immunocompromised, transplant and elderly individuals; as well as individuals with chronic obstructive pulmonary disease (COPD) and asthma. There are approved antiviral medications (e.g. Relenza, Tamiflu) on the market, but these only work if they are given very shortly after onset of symptoms and there is no effective therapeutic treatment for IFV. These studies will be used to evaluate new compounds with the hope of preventing and treating IFV infection. Prior to proposing this efficacy work we conducted a pilot study to determine the peak pathology associated with the viral dose given. Animals were monitored for up to 21 days to monitor weight loss and determine if they began to gain weight and recover from the infection. By day 14 the animals in the 25 $TCID_{50}$ group had generally recovered back to normal, as predicted from studies reported in other publications. By day 10, the 125 $TCID_{50}$ group was euthanized because they hit the 30% weight loss criteria. As we hoped, the 25 $TCID_{50}$ group started to recover on day 10 and returned almost to normal. This provided us with two robust models to test our compounds: a severe acute infection and pathology, as well as a recovery model to test improvements in clinical scores (oxygen saturation and body weight, etc.) over time.

Experimental Objectives. Mice will be infected with IFV by intranasal administration and monitored daily for clinical symptoms of disease, including weight loss and lethargy. New chemical entities (NCEs) will be dosed prophylactically or at various time points post infection to identify therapeutically active compounds that will reduce viral load and/or inflammation. Blood will be drawn at various times after dosing NCEs to verify pharmacokinetic (PK) properties. Mice will be euthanized at time points identified as near the peak of virus replication and/or inflammation to determine the activity of the NCEs in preventing viral replication and/or reducing lung inflammation. Clinical scores, lung inflammation, and viral load will be used as primary endpoints to determine efficacy of the NCEs.

Animals

Nine hundred Balb/c mice will be used.

Animal justification

There are no valid alternatives to the use of animals for studying the full course of IFV disease, including both viral replication and lung immunopathology. The mouse is a highly validated animal model used in IFV research. Whole animal models are necessary for prediction of the effects of the integrated matrix on the analytical outcomes, binding of novel compounds in animals, and interaction with the virus and immune system in vivo. Mice infected with IFV develop significant lung inflammation and viral replication, which closely resembles the intended human patient populations.

Number justification

A typical efficacy study comprises up to 60 animals at a time. Animal numbers for the studies per group/endpoint ($n = 5$–8) have been obtained from the literature, consultation with academic labs, and previous studies using a similar influenza mouse model. Two statisticians with experience designing infectious disease studies have been consulted to determine power calculations for endpoint readouts. Separate mice will be used in each group for lung histopathology and bronchoalveolar lavage fluid (BALF) analysis since we have demonstrated in previous studies that BALF washing the lungs may alter the pulmonary histopathology and scoring. Upon completion of the pilot and initial studies, animal numbers per group will be re-evaluated with a statistician and an update will be given to the institutional animal care and use committee (IACUC) if it is deemed that lower animal numbers will be sufficient. Sixty mice per study × 15 studies per year will equal 900 mice in total. Generally, 3–6 dose groups are used for each study, which depends on the number of doses, compounds, and formulations evaluated within each study. A typical study is designed as follows:

Group 1: Mock + vehicle
Group 2: IFV + vehicle
Group 3: IFV + NCE 1
Group 4: IFV + NCE 2

Fifteen efficacy studies per year are planned.

Assigned methods and procedures

Viral infection

Mice will be identified by ear punch or tail tatoo/marking. For infection, the mouse will first be lightly anesthetized in an isoflurane chamber with 1 L O_2: 3% isoflurane. A drop of the specified volume (50–100 uL) of IFV ($10–2 \times 10^6$ $TCID_{50}$) will be pipetted into the animal's nostril, allowing the drop to be inhaled. The process may be repeated using the other nostril (usually six drops: three per nostril = one dose). Bio Medic Data Systems (BMDS) IPTT300 microchips will be implanted by subcutaneous (SQ) injection to aid in identification and body temperature monitoring, and a drop of tissue adhesive at the trochar injection site will be applied.

Administration of NCEs

Clinical effects. Drug administration, e.g. (oral or parenteral), dosages used will generally be in line with those considered pharmacologically relevant in patients. Previous PK studies will have been performed for tool compounds and other NCEs that will be used for the selection of doses for these efficacy studies, in an effort to minimize the risk of pain or distress to the animals. Regardless of the dose level administered, the animals will be closely monitored for any changes in behavior.

Experimental design. Groups of mice will be infected with IFV by intranasal administration and monitored daily for clinical symptoms of disease, including weight loss and lethargy. NCEs will be dosed prophylactically or at various time points post infection to identify therapeutically active compounds that will reduce viral load and/or inflammation. Blood will be drawn at various times after dosing NCEs to verify PK properties. Animals may be bled periodically during the study period to check for drug concentration levels and inflammatory markers. Typically, mice will be bled by tail nick or tip amputation and blood will be collected in a capillary tube. The blood volume collected will be under 15% in a 24 h period based on a collection

of 1.0–1.8 mL total volume collected from mice in a terminal bleed. Mice will be euthanized at time points identified as near the peak of virus replication and/or inflammation to determine the activity of the NCEs in preventing viral replication and/or reducing lung inflammation. Animals targeted for terminal blood collection and/or tissue collection will be euthanized by exsanguinations under deep isoflurane anesthesia.

Effects of virus infection

- Change in behavior expected to be noted: depression, lethargy, reduced activity, abnormal vocalization, aggression. Influenza-infected mice may become lethargic, display reduced activity, reduce grooming causing ruffled fur, and have labored breathing. Mice will be monitored daily to observe these clinical signs of distress. Mice will be weighed once daily and their activity will be noted once they are taken out of the cage and placed onto a scale.
- Decreased feed consumption that could result in weight loss, lethargy, decreased fecal output, etc.
- Decreased water consumption that could result in dehydration, metabolic imbalance, and decreased urine output. Hydrogel will be provided on the floor of the cages if dehydration is noted.
- A validated clinical scoring system will be used to assess mice daily.
- Daily body weight will be taken. Weight loss (10% or more) or thin body condition (score 2/5 or less) will result in nutrient gel being added to the floor of cage.

Humane endpoints

- Cardiovascular disease with related clinical signs (e.g. coughing, respiratory distress, cyanosis, limb edema).
- Hunched posture in conjunction with other clinical signs and especially if debilitating or prolonged (3 days).
- Inability/unwillingness to ambulate to reach food or water.
- Marked changes in behavior noted: severe depression, non-responsiveness, listless, unwilling to move.
- Other clinical signs judged by experienced technical staff to be indicative of morbidity or being in a moribund condition.
- Weight loss of up to 30% is anticipated. We are requesting IAUC permission to keep mice alive for up to 30% weight loss, instead of 20%, so we can monitor the full course of infection and disease and allow for therapeutic treatment of lung inflammation. Publications using the strains of IFV in this protocol have documented weight loss of up to 35% with higher doses of virus, but we plan to use lower doses and keep weight loss at 30% or below. Mice also have been documented to recover body weight over time if they are monitored for up to 21 days. We aim to determine an appropriate course of disease to induce significant lung inflammation and virus replication to allow for therapeutic treatment with NCEs, but without causing severe disease or weight loss of $>30\%$.

Housing and husbandry. Mice will be housed in microisolator caging, three per cage, in the pharmaceutical company vivarium. Cages will be provided with nesting material and plastic huts for warmth and environmental enrichment. The ambient temperature of the room will be raised and controlled at $75 \pm 2^{\circ}$F. This is an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accredited animal care and use program. The rodent housing facility is excellent and supported by an experienced (>5 years average) animal care staff, all of whom are certified by American Association for Laboratory Animal Science (AALAS).

Appendix Table 1. A suggestion of harm-benefit analysis for the mouse example using the WG's suggested model.

HARM TABLE (mouse protocol)

HARM-Freedoms Impacted
Pain/Injury: Severe flu infection
Fear/Distress: respiratory distress
Hunger/Thirst: NA
Ability to express normal behavior: NA
Discomfort/Husbandry: Daily manipulation

Modulating Factors for HARM	Description	Mitigating Effect	Aggravating Effect	Summary Color
Animal— Species	Mouse	Highly adapted to laboratory environment/low societal concern		
Animal— Number	900	Statistically justified/reviewed		
Animal— Suited to environment	Laboratory reared			
Animal— Health status	Pathogen free			
Experimental- Intensity	Mice infected with influenza	Treat dehydration/ anorexia with fluids and mash/		
Experimental Duration	21 days – ill for up to 14 days			
Experimental Cumulative Experience	One experiment/life span			
Experimental—Endpoint	30% weight loss	Clinical scoring/body weights/temperature monitoring occurring daily		
Experimental Complication/Distribution Rate	Bacterial secondary pneumonia	Humane endpoints described /low incidence		
Experimental Phenotypic Modulation	Respiratory Distress	pulse oximetry to monitor P02- heads up on end point signaling		
Environmental Housing/Husbandry	Social housing with environment enriched			
Environmental Personnel competence/experience	Highly experienced scientific and husbandry team with mice and with model			

BENEFIT TABLE (mouse protocol)

BENEFIT DOMAINS
Social
- Human health: Important unmet need
- Animal health: NA
- Environment health: NA

Socioeconomic: Large/keep people out of hospital
Scientific: Proprietary
Educational: NA
Safety and Efficacy: NA

Modulating Factors for Benefit	Description Why/How/What /When	Summary Color/ Numeric Score
Importance of outcome	Why= important unmet medical need	
Clarity of objectives	How/What= objectives crisp –find new drug	
Translational Potential	Who= mouse has potential/not proven translation ability	
Likelihood of success	How= Novel drug, only tested in cell culture	
Continuity of recognized scientific efforts	What= further advances the knowledge base of drug development	
Quality of Experimental Design	How= follow up to pilot study that were successful /robust reproducible model	
Innovation Level	How= novel drug- new mechanism of action (model not innovative)	
Dissemination of Results	When/How=proprietary	

Protocol Example 2: Longitudinal left ventricular remodeling following myocardial infarction

STUDY OBJECTIVES

The development and progression of congestive heart failure has reached epidemic proportions worldwide. It is estimated that currently over 10 million patients suffer from this condition. One of the common causes for congestive heart failure are the long-term effects of a heart attack – myocardial infarction (MI). Despite significant advances in our abilities to reopen and restore blood flow to the heart muscle, methods to prevent the long-term effects of the damaged myocardium have not been forthcoming. A structural milestone in the development and progression of congestive heart failure secondary to MI is myocardial remodeling. This is defined as changes in left ventricular (LV) geometry and structure which in turn can reduce pumping efficiency. It is now recognized that the region of the myocardium surrounding the MI changes shape and size and that this in turn is translated into overall changes in LV geometry. This phenomenon is termed 'infarct expansion' and has been identified as an important therapeutic target to minimize post-MI remodeling, subsequent LV remodeling, and in turn reduce the progression to heart failure. Exacerbated infarct expansion in the early post-MI period has been hypothesized to be an independent predictor for accelerated LV dilation and, potentially, progression to the development of heart failure post-MI. Accordingly, the goal of this study will be to longitudinally measure regional and global LV geometry in the same post-MI pigs to develop a relationship between early regional changes in infarct geometry (regional infarct expansion) and later increases in LV dimensions (global LV remodeling).

This study will use MRI in a well-established porcine MI model to develop a temporal relationship between regional and global changes in LV geometry in the same pigs post-MI, we believe we will be contributing new information using MRI.

RATIONALE FOR ANIMAL USE

1. *Explain your rationale for animal use.*
   There are no in vivo models that simulate regional and global remodeling in the LV following MI. Therefore, there are no alternatives to performing these procedures in animals.
2. *Justify the appropriateness of the species selected.*
   Pigs have been shown to be an excellent model for performing studies to determine changes in the myocardial extracellular matrix (ECM) in a number of simulated cardiac disease states. Importantly, it has been demonstrated that pigs most accurately reflect the coronary anatomy of humans and respond in a similar fashion to myocardial ischemia/infarction. Secondly, pigs can be obtained in consistent sizes and weights and therefore, reducing variability between experimental observations.
3. *Justify the number of animals to be used.*
   Power analysis indicates 10 animals in the non-MI group for Phase 1 and 10 animals in the MI group for Phase 2. We anticipate a mortality rate of 20% so will assign 13 animals per group with the studies repeated in triplicate. A total of 87 animals will be used, 29/year/3 years.

EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES

Pigs will be allowed to acclimatize within university facilities for a minimum of seven days. For the first five days that the pigs are in-house, all pigs will be administered erythromycin (250 mg PO, TID) due to their conventional health background. On the day prior to instrumentation, on the day of instrumentation, and the day following instrumentation, the pigs will be administered Naxcel (3.0 to 5.0 mg/kg, intramuscularly).

Surgical modeling

For control animals, a purse string will be made in the thoracic aorta and a catheter connected to an access port will be advanced to the aorta at the level of the diaphragm. The access port connected to the catheter will be placed in a subcutaneous pocket and secured in place using silk ties. For MI-induced animals, a pericardiectomy exposing the LV free wall, the left atrium, the circumflex artery and the obtuse marginal (OM) branches will be performed. A purse string will be made in the thoracic aorta and a catheter connected to an access port advanced to the aorta at the level of the diaphragm. The access port connected to the catheter will be placed in a subcutaneous pocket and secured in place using silk ties. OM arteries from the circumflex coronary artery will be identified. Ligatures (Proline 4.0) will be placed around the origins of OM1 and OM2. MI will be induced by permanent ligations of OM1 and OM2. Both control/MI pigs will be imaged at 28, 42, and 56 days post-surgery and terminally studied after imaging at 56 days post-surgery.

Serial imaging measurements

Sedation and anesthetic induction. Animals will be sedated with benzodiazepam mixed in a food administered 2 h prior to study, and placed in a custom-designed sling that will allow the animal to rest comfortably. The animals will be intubated with a cuffed intubation tube and will be allowed to self-ventilate with 0.5–3.0% isoflurane delivered through a portable anesthesia machine. Heart rate and rhythm will be continually monitored using surface electrocardiogram (ECG) recordings.

The skin over the vascular access port on the back will be shaved and prepared in a sterile fashion with alternating wipes of betadine and alcohol. The access port will be entered with a custom needle (Huber) and drawback of arterial blood confirmed. The access port with the associated intravenous line will then be capped using sterile supplies and housed in a custom-designed pouch.

Imaging. Longitudinal measurements of LV geometry and function will be performed using MRI. As an additional measure of perfusion, gadolinium (Gd) enhanced contrast MRI images will be recorded.

Recovery. Following the completion of imaging studies, the animals will be transported back to their housing facilities. The access port will then be flushed with heparinized saline (1000 U/mL) supplemented with cefazolin. The Huber needle will be removed. The animals on which future imaging studies will be performed will be weaned off anesthesia, extubated, and then returned to cages once recovered from anesthesia. The animals in which the final set of imaging studies are completed, will be processed for terminal studies.

Terminal studies. Following the final set of MRI studies at 56 days post-MI, the pigs will be anesthetized for assessment of global and regional LV functions, microdialysis measurements and hemodynamics. Anesthetic induction will commence using isoflurane (3%) in a mixture of oxygen and nitrous oxide (67:33%, 1.5 L/min) delivered by face mask. Once the pig is adequately anesthetized, peripheral venous access (ear) will be obtained and a 2 μg/kg dose of sufentanyl (ESI) will be injected through the ear vein cannula. A 0.1 mg/kg dose of etomidate and a 10 mg dose of vecuronium will then be administered intravenously after ensuring that the animal remains adequately anesthetized. This results in a rapid deepening of the already established surgical plane of anesthesia. An endotracheal tube will be surgically placed via a midline submandibular incision and mechanical ventilation established. Anesthesia will be maintained throughout the procedure by delivery of 0.5% isoflurane and intravenously administered morphine (ESI; 3 mg/kg/h). The delivery of isoflurane, nitrous oxide and morphine will be titrated to maintain stable physiological hemodynamic and respiratory profiles. Intensive and continuous monitoring of various vital signs will provide the necessary means to ensure a complete and stable surgical plane of anesthesia. Following stabilization of this surgical anesthetic plane, an intravenous infusion of vecuronium (15 mg/h) will be initiated. This infusion will be titrated as needed to provide continuous muscle relaxation which facilitates appropriate mechanical ventilatory control and a stable surgical field. Additional 5 mg boluses of vecuronium will be administered as needed to support these goals. The heart rate and blood pressure will be carefully monitored to ensure that the animals remain at a stable surgical plane of anesthesia throughout the procedure.

A multi-lumen thermodilution catheter will be positioned in the pulmonary artery via the right external jugular vein. An 8 F introducer with a side-arm will be placed in the right carotid for blood pressure measurements and arterial access. The aortic access port will also be connected to monitor systemic pressures. A Foley bladder catheter will be surgically placed and secured via a midline suprapubic retroperitoneal incision. A sternotomy will be performed and a vascular ligature will be placed around the inferior vena cava in order to perform transient caval occlusion. A previously calibrated microtipped transducer will be placed in the LV through a small apical stab wound. Piezoelectric crystals will be positioned in the LV endocardium in order to provide an orthogonal myocardial dimension across the short axis in two regions: the MI region and the remote region. The remote regions will be defined as the area served by the left anterior descending artery (LAD).

The terminal procedure is expected to take place over an 8 h period.

OTHER

- **Transportation**
  For imaging studies, animals will be transported to the magnetic resonance imaging (MRI) facility in an enclosed van. Animals will be loaded and unloaded from the vehicle in such a manner that visibility to the public will be minimized through careful selection of transport route and utilization of enclosed loading bays. This vehicle will be specifically outfitted to transport the sling and anesthesia machine (with associated monitoring equipment) in a locked position so that the animals being transported remain stationary relative to the vehicle. In addition, a high capacity power inverter will be installed to provide power to the anesthesia machine and

monitoring equipment while in the transport van. For terminal studies, animals will be transported to the investigators laboratory in a large mobile animal crate covered with a sheet.

- **Animal identification methods**

  Pigs will have ear tags placed upon arrival.

- **Methods of restraint**

  Pigs will be restrained in a pig sling for a period of up to 60 min. This is not considered prolonged, and the pigs appear comfortable in the sling. In our experience, no acclimatization is necessary.

- **Experimental injections or inoculations**

  Gd-DTPA will be manually injected at a dose of 0.1 mmol/kg through the vascular access port for imaging.

- **Blood withdrawals**

  In order to provide an estimate of MI size, an aortic blood sample will be drawn from the subcutaneous access port. The pig will be placed in a custom-designed sling that will allow the animal to rest comfortably in a non-restrained fashion. The area around the access port (5 cm) will be washed and prepped and a sterile field created. The access port will be entered with a custom needle (Huber) and 3 cc of aortic blood drawn. The access port will then be flushed with heparinized saline (1000 U/mL) supplemented with cefazolin. This entire procedure will last approximately 30 min. Throughout the 56-day study period, additional 3–5 cc blood samples will be collected weekly to assess clinical chemistry and complete blood count (CBC) parameters.

- **Food or fluid restriction**

  Animals will be fasted for 12 h prior to the surgery.

- **Pharmaceutical-grade and non-pharmaceutical-grade compounds**

  Not available.

- **Resultant effects**, if any, that the animals are expected to experience.

  Animals are expected to experience hemodynamic compromise that may result in inappetance, inmobility, weight loss, vomiting, diarrhea. Pain and distress are expected with the surgical procedures.

- **Other potential stressors** [*e.g. noxious stimuli, environmental stress*] **and procedures to monitor and minimize distress**

  The access port can create a level of discomfort/distress for the pig. We will evaluate its patency weekly, and evaluate daily for signs of infection.

- **Experimental endpoint criteria**

  The animals will be pulled from the study and euthanized if their weight loss exceeds 20% of their starting weight.

- **Veterinary care**

  Every surgically-altered animal involves a large resource commitment, so every effort will be made to address clinical issues that arise with the vascular access port, hemodynamic compromise or other issues. Investigators will consult with staff veterinarians for a treatment plan.

SURGERY

If surgery is proposed, complete the following:

1. *Identify and describe the surgical procedure(s) to be performed. Include preoperative procedures* [*e.g. fasting, analgesic loading*], *and monitoring and supportive care during surgery. Include the aseptic methods to be used.*
   The animals will be fasted for 24 h, and on the morning of surgery, a 100 μg fentanyl patch (5 μg/kg/day, release rate of 50 μg/h) will be applied in addition to an intramuscular injection of buprenorphine (0.05–0.1 mg/kg). A surgical plane of anesthesia will be provided through the use of inhalation isoflurane. Just prior to surgery, anesthesia will be induced with ketamine (22 mg/kg), acepromazine (0.04 mg/kg), and atropine (0.04 mg/kg) by trained staff and the pigs will be placed in a custom-designed pig sling. An ear vein will be accessed and the venous cannula left in place to administer intravenous fluids (e.g. lactated Ringer's) and other pharmacological agents (e.g. antiarrhythmics, such as lidocaine) if needed. An ECG and pulse oximetry will be established. Anesthetic induction will also be established using a face mask delivering isoflurane (3%, 1.5 L/min) and nitrous oxide (0.5 L/min). The animal will then be intubated with a cuffed endotracheal tube and ventilated at a flow rate of 22 mL/kg/min. Regulation of the delivery of isoflurane will be used to maintain a stable heart rate

and blood oxygenation, and will be increased if either of these parameters rises by over 10% from ambient levels. Oxygen saturation and heart rate will be monitored continuously to provide a sensitive means to ensure a complete and stable surgical plane of anesthesia. A lidocaine infusion will be initiated with a 3 mg/kg bolus followed by a constant infusion of 120 mg/h.

2. *Identify the individual(s) who will perform surgery and their qualifications, training, and/or experience.*
   Dr Expert's fellow will be performing the surgeries, initially under the direction of Dr Expert, but practice, independently. Dr Expert is a trained cardiothoracic surgeon who will be getting training in this particular procedure in pigs from Dr Supreme, a veterinarian surgeon experienced in CV procedures.
3. *Identify the location where surgery will be performed.*
   Dr Expert has her own surgical facilities in Building D and surgeries will be conducted in that location.
4. *If survival surgery, describe postoperative care that will be provided and frequency of observation. Identify the responsible individual(s) and location(s) where care will be provided* [*building(s) and room(s)*].
   Include detection and management *of postoperative complications during work hours, after hours, weekends and holidays.*
   The animal will be recovered from the surgery in an intensive care unit under the direction of the staff veterinarians. Buprenorphine 0.005–0.02 mg/kg will be utilized for immediate post-surgical pain. For prolonged post-thoracotomy pain and more prolonged analgesia, ketoralac tromethamine will be administered.
5. *If non-survival surgery, describe how euthanasia will be provided and how death will be determined.*
   Following completion of the protocol described above, isoflurane delivery will be increased to 5%, and maintaining full anesthesia, cardioplegic arrest will be induced through delivery of a 24 mEq potassium solution in lactated Ringer's through the aortic root. The heart will be harvested and the LV isolated and placed in chilled Krebs solution.
6. *Are paralytic agents used during surgery? If yes, please describe how ventilation will be maintained and how pain will be assessed.*
   Pancuronium (15 mg/h) will be administered for the terminal procedures. The pig will be on a mechanical ventilator and blood pressure, heart rate and oxygen levels will be continuously monitored.
7. *Has major or minor survival surgery been performed on any animal prior to being placed in this study? If yes, please explain.*
   No
8. *Will more than one survival surgery be performed on an animal while in this study? If yes, please justify.*
   No

PAIN OR DISTRESS CLASSIFICATION AND CONSIDERATION OF ALTERNATIVES

1. Pain or distress classification for USDA covered species.

   USDA Classification D = Animals subjected to potentially painful or stressful procedures for which they receive appropriate anesthetics, analgesic and/or tranquilizer drugs.

2. Consideration of alternatives:

A Pub Med search from 2012–2015 using the keywords ventricular remodeling, pig, animal alternatives indicated that alternatives were not available.

ANESTHESIA, ANALGESIA, TRANQUILIZATION, OTHER AGENTS

Analgesics

Perioperative analgesia: 100 ug fentanyl patch (5 ug/kg/day); intramuscular injection of buprenorphine (0.05–0.1 mg/kg/ im).

Post-operative analgesia: beuprenorphine 0.005–0.02 mg/kg. Long-term discomfort: Ketoralac at veterinary recommended dose.

Anesthesia

Ketamine (22 mg/kg), acepromazine (0.04 mg/kg), atropine (0.04 mg/kg), isoflurane, nitrous oxide.

METHOD OF EUTHANASIA OR DISPOSITION OF ANIMALS AT END OF STUDY

Pigs will be euthanized by exsanguination under a surgical plane of isoflurane anesthesia. Animals deemed to be in distress will be euthanized by a barbiturate overdose. These methods are consistent with the recommendations of the Panel of Euthanasia of the AVMA.

EXEMPTIONS FROM ENVIRONMENTAL ENRICHMENT AND SOCIAL HOUSING

Previous work has shown that the externalized instrumentation requires individual housing, the pigs cannot be housed socially. Enrichment devices will be provided as determined by the veterinary staff.

PRINCIPAL INVESTIGATOR CERTIFICATIONS TRAINING

1. I certify that I have attended the institutionally required investigator training course.

   Year of Course Attendance: *20xx*

   Location: *Certified Laboratory Animal Training Center*

   Additional Training: Board certified Cardiothoracic surgeon

   Additonal Training: Board Certified Veterinary Surgeon

Appendix Table 2. A suggestion of harm-benefit analysis for the pig example using the WG's suggested model.

HARM TABLE (pig protocol)

HARM-Freedoms Impacted
Pain/Injury: Survival Surgery
Fear/Distress: Transport/ Imaging/ Surgery
Hunger/Thirst:
Ability to express normal behavior: Single housing
Discomfort/Husbandry: Surgical Port with Catheter

Modulating Factors for HARM	Description	Mitigating Effect	Aggravating Effect	Summary Color
Animal— Species	Pig-		Non rodent model/USDA covered species - more societal concern	
Animal— Number	87		large number of non-rodent in single experiment	
Animal— Suited to environment	Farm raised		Not acclimated to laboratory conditions	
Animal— Health status	Farm raised		Potential confounding pathogens/factors	
Experimental- Intensity	Survival Surgery/ imaging/ indwelling ports /catheters	Conscientious use of anesthetics/analgesics	Complex survival surgery/instrumentation throughout study	
Experimental Duration	60 days	Short time frame		
Experimental Cumulative Experience	Single experiment, but multiple manipulations	Single experiment	Multiple experimental manipulations	
Experimental—Endpoint	Weight loss	Clearly defined	Spectrum of morbidity	
Experimental Complication/Distribution Rate	20% mortality	Complex model- not excessive mortality-state of art		
Experimental Phenotypic Manipulation	Ventricular damage	Supportive clinical care allowed		
Environmental Housing/Husbandry	Single housing Animals experience transport stress	Pen enrichment provided, interaction with technicians, staff well trained on use of pigs	Pigs are social animals	
Environmental Personnel competence/experience	Surgeon not trained in 'procine surgery'	Will be working with trained porcine surgeon		

BENEFIT TABLE (pig protocol)

BENEFIT DOMAINS
Social
- Human health: Meets strong unmet medical need
- Animal health: Information useful for unmet animal health need
- Environment health: NA

Socioeconomic: Health care costs significant
Scientific: Cellular mechanism illucidation
Educational: NA
Safety and Efficacy: NA

Modulating Factors for Benefit	Description Why/How/What /When	Summary Color/ Numeric Score
Importance of outcome	Why= Strong unmet medical need for animals and humans	
Clarity of objectives	How/What= Objectives clearly stated	
Translational Potential	Who/When= Pig excellent CV model with proven translational results in model	
Likelihood of success	How= very complicated model	
Continuity of recognized scientific efforts	What=important existing knowledge base of CV disease in pigs , adds to this	
Innovation Level	How=funded by NIH , support of level of innovation	
Quality of Experimental Design	How= NIH funded, establishing model of CV disease	
Dissemination of Results	When/How= Publications detailing results and methods component of NIH funding	

NIH: National Institutes of Health