

HANDBOOK OF LABORATORY ANIMAL SCIENCE

Essential Principles and Practices

FOURTH EDITION



EDITED BY
JANN HAU
STEVEN J. SCHAPIRO

Handbook of Laboratory Animal Science

Fourth Edition

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CHAPTER 1

Animal Research Ethics

Anna Olsson and Peter Sandøe

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INTRODUCTION

In March 2012, less than 2 years after agreement had been reached on a new European directive regulating animal research, a European Citizens' Initiative calling for the directive to be abrogated was launched. In 2015, after gathering the necessary support (1 million signatures in total and a minimum of seven EU countries), Stop Vivisection were invited to a public hearing in the European Parliament. The demand they made was simple: from 2021 "animal experiments made for the knowledge of human responses have to be outlawed" (Stop Vivisection 2015). Thus, although the legal standards incorporated in the European directive are among the most demanding in the world, they are still viewed by some as not going anywhere near far enough.

As a proposal, complete abolition of animal experiments within 5 years seems straightforward. But

examination of the arguments behind the Stop Vivisection initiative shows that the background to this proposal was complex. The overall strategy of Stop Vivisection appealed primarily to scientific aspects of animal use in biomedical research. The group argued that animal experimentation is not only not useful for human health research but actually directly harmful as a result of its tendency to generate misleading findings. However, since the central function of a Citizens' Initiative is to bring an existing piece of legislation into discussion, Stop Vivisection also had to explain why Directive 2010/63/EU was not an appropriate instrument for regulating the use of animals in research. And here the argument about usefulness seems to have played a limited role. Instead, the directive was criticized for giving primacy to economic interests, disallowing member states from introducing more stringent legislation, allowing the use of primates and stray cats and dogs

in research, allowing reuse of animals, allowing procedures without anesthesia when this is scientifically justified, not making nonanimal alternatives mandatory, and including a simplified administrative procedure for licensing certain standard projects.

As can be seen from this example, the discussion of animal research ethics is complex. In this chapter, we will address the ethical issues at stake in animal experimentation in a way that helps to clarify the underlying issues.

We start by presenting an argument used by some of those who oppose animal experimentation: the idea that it is wrong in principle to use animals in experiments that cause them harm. We will discuss how this argument is positioned within a wider context of ideas about how animals ought to be treated: the animal rights view. We will go on to look at other ideas about animal ethics from a theoretical perspective and will conclude the section on animal ethics views by reviewing what is known about public perception on animal use in experiments. Although there is increasing public concern over animal suffering and the killing of animals, there does not seem to be widespread public support for a ban on animal use in experiments. However, there is support for the view that animal experimentation should only take place if certain conditions are met. In the second half of the chapter, we will present and discuss some of the conditions that have been proposed. We will first present the more traditional view of animal research ethics as a matter of maximizing benefit and minimizing harm. We will then discuss how additional requirements, including harm–benefit weighing and the setting of limits on animal suffering, are making their way into frameworks guiding animal research ethics. We will finish the chapter with a section on mechanisms for setting and upholding standards.

THE ARGUMENT THAT ANIMAL EXPERIMENTATION IS WRONG IN PRINCIPLE BECAUSE IT INVOLVES THE DELIBERATE INFILCTION OF HARM ON ANIMALS: ANIMAL RIGHTS AND RELATED VIEWS

The basic concern that animal experimentation is wrong because it involves the deliberate infliction of harm on animals has been important since at least the nineteenth century, when the early anti-vivisection movement emerged. This was a time when effective anesthesia was not available for humans or other animals. Animals used in physiological research were sometimes cut open and experimented on while alive (hence the term “vivisection”), often when they were fully conscious. At the same time, most of the research done with animals was of a very basic sort, which served to generate new knowledge but had little direct impact on efforts to cure diseases or improve human health.

Since then, there have been improvements in our ability to anesthetize animals and limit their pain and suffering by means of analgesia. The connection between animal experimentation in biomedical research and the development of drugs, vaccines, and other means of preventing, curing, or alleviating human disease has also become much closer. So, pain and other harms imposed on animals during experimentation are much better controlled today than they were in the early days when there was no legislation, and the potential benefits of animal use seem much more obvious today than they once were. However, this is not to say that there is no moral problem.

Animals still suffer in numerous ways during animal experimentation, partly because not all suffering can be controlled, and partly because, to achieve their goals, some experiments require the animals not to be medicated. And in nearly all experiments, the animals are killed at the end, which, even when it is painless, can still be viewed as a harm to the animal. In light of this, some may conclude that animal experimentation is morally unacceptable because it is wrong *in principle* to inflict harm on living animals.

Some people who support this line of argument may try to downplay the benefits of research with animal models. Others, who object to the argument, may overstate the benefits of animal experiments. However, there are also those who argue that benefits are irrelevant here because animals have inviolable rights that are inevitably violated in animal-based research.

Thus, the American philosopher, Tom Regan, a vocal advocate for animal rights, argues like this: “The rights view is categorically abolitionist. Lab animals are not our tasters; we are not their kings. Because these animals are treated routinely, systematically as if their value were reducible to their usefulness to others, they are routinely, systematically treated with lack of respect, and thus are their rights routinely, systematically violated. This is just as true when they are used in trivial, duplicative, unnecessary or unwise research as it is when they are used in studies that hold out real promise for human benefits” (Regan 2007, p. 210).

The main point of moral rights of the kind defended by Tom Regan is to define boundaries that may not be crossed (unless, of course, the holder of the right waives it, which is academic if we are talking about animal rights). We will soon clarify in more detail what rights animals may be considered to have, but even without this clarification, one can see the appeal of a rights theory. The attribution of rights to animals allows us to insist that some ways of treating animals are totally unacceptable—not unacceptable if enough people disapprove or if the benefits are too small—but unacceptable, period (see also Regan 1984).

In Regan’s view, human practices, such as using animals in invasive research and keeping animals for meat,

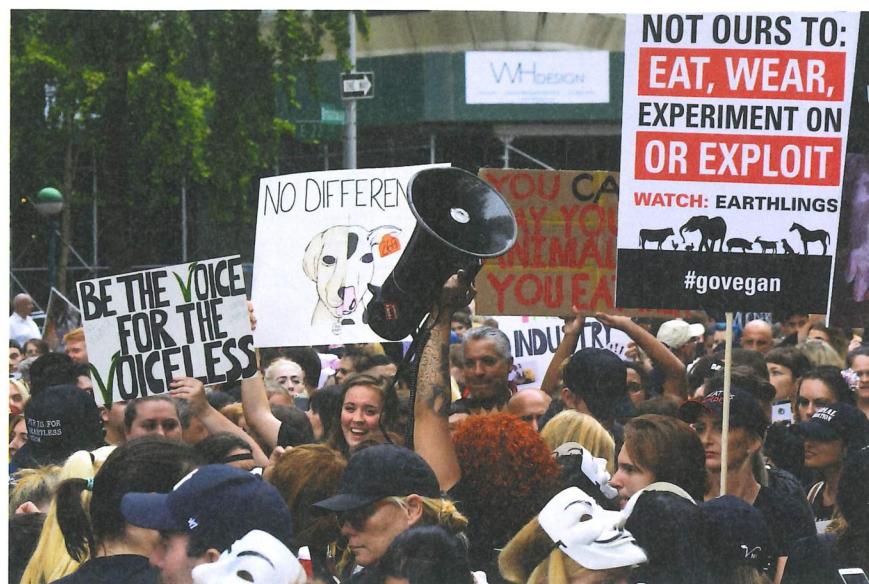


Figure 1.1 Protesters from Anonymous for the Voiceless demonstrating against the use of animals. (Image by Robert Jones from Pixabay.)

are morally unacceptable; they violate the animal's right not to be harmed, including its right not to be killed. This does not necessarily imply that all forms of animal use should be banned. In his *Animal Rights without Liberation* (2012), Alistair Cochrane develops an interest-based view of animal rights in which core animal interests, such as not to be made to suffer and not being killed, are protected by rights. But he maintains that since animals do not understand what "being property" is, they do not have an interest in avoiding some forms of animal use, such as being kept as pets or as zoo animals, or participating in noninvasive experiments within, for example, behavioral studies.

But even if some interpret animal rights in a way that makes them compatible with some human ways of keeping animals, all animal rights proponents will reject the mainstream use of animals in biomedical research because (1) during the experiments, nontrivial physical harms are imposed on the animals and (2) most animals are killed after the experiment. In most cases, the animal rights advocate will take an uncompromisingly abolitionist position on animal experimentation as we know it today.

Understood in broad terms, the animal rights perspective includes recent attempts to consider animals as co-citizens with political rights [see Donaldson and Kymlicka (2012) and Arnason (2017)]. Adherents of this view favor the banning of nearly all of the ways in which humans presently use animals. To many, this view will sound radical, but its proponents are likely to argue that this is because society has not yet caught up with the idea that animals have rights that we must respect. For now, the fact

that animal experimentation is not socially condemned by the majority of people suggests that animal rights-based thinking is not widespread in society. Indeed, this has been confirmed in empirical research, as we will see in the next section.

PUBLIC PERCEPTION: WIDE SUPPORT FOR THE USE OF ANIMALS IN RESEARCH AND TESTING

People disagree over the moral acceptability of using animals for research and testing. Studies undertaken in Denmark (Lund et al. 2012, 2014) identify three kinds of stances among members of the Danish public: dis approvers (16%) hold the view that research with animals cannot be accepted despite potential benefits to humans; reserved people (49%) are ambivalent and typically shift between approval and disapproval of specific experiments depending on harm–benefit evaluations; and approvers (35%) tend to put more weight on the potential human benefits than they do on protecting animals from suffering. Another relevant result of these studies is that most people seem to accept the harm–benefit framework as a basis for making decisions about the use of animals in research and testing.

There seem to be large national variations, both regarding general acceptance of animal use and regarding the use of particular species. A comparative study done in 28 EU countries comparing attitudes on the use of mice to the use of dogs and monkeys in animal experimentation (von Roten 2013) showed three things: (1) generally, experiments on mice are more accepted than experiments

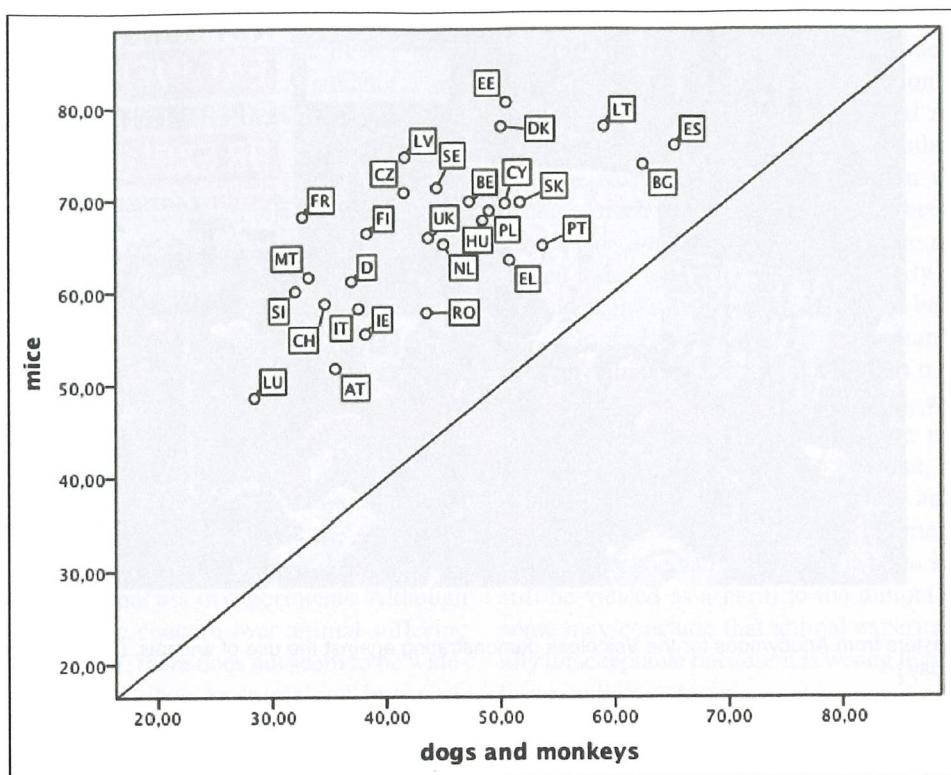


Figure 1.2 Relation between percentage of acceptance of experimentation on mice and on dogs and monkeys among 28 EU countries in 2010 (in %). (From von Roten, Fabienne Crettaz, *Public Understanding of Science* 22 (6): 691–703, Copyright © 2013 by SAGE Publications. Reprinted by Permission of SAGE Publications, Ltd.)

on dogs and monkeys; (2) differences in the acceptance of mouse use and the use of dogs and monkeys vary considerably across countries; and (3) there are dramatic differences in the levels of acceptance across countries, ranging from around 30% to around 70% in the case of dogs and monkeys, and from around 50% to around 80% in the case of mice (Figure 1.2).

Similar results on public acceptance of animal experimentation are seen when individual studies are compared. Thus, a recent study from the UK (Ipsos MORI 2018) found an acceptance rate of 65%, whereas studies from Italy (Speaking of Research 2014) and the United States (Pew Center 2015) found acceptance rates of 49% and 47%, respectively. However, some caution is called for when comparing these numbers. A lot may depend on how questions are asked. Thus, in the UK study, people were asked whether they could accept the use of animals in research “as long as there is no unnecessary suffering to the animals and there is no alternative,” whereas in the other two studies they were asked simply about use of animals “for medical research” and “for research.”

Still, the general picture is that in most countries, there is a clear majority in favor of using animals for important biomedical research, but the level of

acceptance drops if animals are used for less important purposes and if the animals have to endure significant suffering. At the same time, there are generally higher levels of acceptance among men than there are among women and among older persons than younger persons. Also, when acceptance is measured longitudinally, as happened in the U.S. study, a drop in acceptance is observed over time. So, although there is still support for the use of animals in vital biomedical research, there is a trend: concern about the suffering imposed on animals, the species used, and the lack of genuine benefit from research is growing.

THE ARGUMENT THAT ANIMAL EXPERIMENTATION IS ACCEPTABLE, IN PRINCIPLE, IF THE RIGHT BALANCE BETWEEN HARM AND BENEFIT IS STRUCK: UTILITARIANISM AND ANIMAL WELFARE

As we saw, the majority of the general public are concerned about animal experimentation and testing due to the suffering that may result. On the other hand, many people also accept some form of animal use, provided that harms to animals are limited and as long as

there are genuine benefits to human health or other vital rewards to be gained. This mixed view has important similarities to the other main contender, besides animal rights, in ethical discussions concerning animal use: utilitarianism.

Avoidance of suffering is a key moral concern for adherents of so-called *utilitarian ethics*. Famously, the founder of this school of thought, Jeremy Bentham, included animals in the realm of morality precisely because they have the ability to suffer. “The question is not, Can they reason? nor, Can they talk? but, Can they suffer?” (Bentham 1789/1989). According to the utilitarian, morality has one basic rule: always act so as to maximize the well-being of those affected by your actions. The good to be maximized, *well-being*, is usually defined in terms of enjoyment and the absence of suffering. For the modern utilitarian, as for Bentham, all well-being matters in exactly the same way, whether it belongs to a concert pianist or a pregnant sow. In this sense, all sentient creatures, human and nonhuman, deserve equal moral consideration. There will be plenty of situations in which we can improve the well-being of animals in our care at little cost to our own welfare. When these situations arise, we have a moral obligation to attend to the animals’ interests because we have a moral obligation to act always in ways that maximize well-being. In this sense, animals make genuine moral demands on us.

From the utilitarian approach, then, ethical decisions require us to strike the most favorable balance between benefits and costs for all of the sentient individuals affected by what we do. This is uncontroversial when the balance can be achieved without excessive cost to human stakeholders. But in contemporary western society, we retain a general tendency to give ourselves priority over animals in a way that a utilitarian will regard as essentially wrong. There are many examples where relatively trivial human interests are treated as if they overrule much stronger animal interests. The human interest in cheaper, time-effective production systems for meat and other animal products prevails, despite substantial evidence that the welfare of farm animals could be improved immensely at limited cost.

In the ethical debate over animal research, the tension is sometimes between strong interests on both sides. Utilitarianism, as described earlier, suggests that animal interests can be sacrificed where such sacrifice leads to the protection or satisfaction of human interests that are strong enough to outweigh the animal interests at stake. However, often experimentation means sacrificing vital animal interests in continued life and the avoidance of abject suffering where it is not obvious that these can be outweighed by the human interests involved.

Insisting firmly that human and animal interests deserve equal consideration, the utilitarian philosopher Peter Singer has concluded that the sacrifice of such vital

animal interests is acceptable only where the benefits are extraordinarily important:

... if a single experiment could cure a disease like leukemia, that experiment would be justifiable. But in actual life the benefits are always much, much more remote, and more often than not they are non-existent.

(Singer 1975, p. 85)

So, even though Peter Singer would in principle accept animal experimentation, in practice he finds most currently undertaken animal research and testing unacceptable. In practice, the significant harm imposed on the animals is substantial, and it is not offset by the benefits to humans that can be expected to come out of the experiments.

While the majority of people hold a view like the utilitarian one held by Singer, in which both harms to animals and human benefits matter morally, they do not go so far as to consider animal and human interests equivalent. Thus, in present moral practice, there is a built-in assumption that nontrivial human interests supersede animal interests, whatever the cost to animals. The political scientist Robert Garner has dubbed this view, according to which human and animal interests belong to different orders of moral worth, “animal welfarism” (Garner 2017).

Clearly, this is not a stable situation. As indicated earlier, the public view is developing toward increasing emphasis on preventing animal suffering and the unnecessary killing of animals. This, as we shall see in the next section, is reflected in the ethical norms guiding animal experimentation and the legislation underpinning it. While the thinking that dominated the field until about 20 years ago was fully in line with animal welfarism, and therefore, essentially accepted all experiments to the extent that they served decent purposes provided that efforts were made to limit animal suffering as much as possible, this is no longer so clear.

SETTING THE STANDARDS FOR ANIMAL RESEARCH

This chapter so far has looked at different sets of arguments around how animals ought to be treated. We have seen that there is a diversity of views among ethicists, as well as in public opinion. Although this means that it will be impossible to find an approach that is generally satisfactory, it remains necessary to find a compromise on which standards can be based. The requirements to minimize animal suffering and to pursue decent and well-founded research goals seem to play an important role in determining whether animal use is considered acceptable in a given research context. This translates roughly into

the aims of limiting any harm caused to the animals and increasing any benefit to be derived from proposed experiments. This is the approach we examine first. After that, we will look at approaches that challenge the status quo by requiring that harms may only be imposed on animals if they are justified by proportional expected benefits or that there should be an absolute cap on the suffering imposed on animals.

The Traditional Approach: Maximizing Benefit and Minimizing Harm

What Are Benefits and How Can They Be Maximized?

Broadly speaking, the aim of animal research is to secure benefits—chiefly through the acquisition of new knowledge that provides answers to fundamental questions in biology or improves human and animal health and safety. However, as many animal researchers will ruefully confirm from personal experience, the assumption that benefits will be delivered cannot be taken for granted. Science is not a predictable “manufacturing” activity, and even when we are armed with well-defined questions and correctly designed and carefully executed experiments, it is sometimes impossible to predict whether a research project will improve our understanding of important biological mechanisms or lead to the development of useful applications.

Traditionally, the assessment of benefits has been based upon measures of scientific potential and quality. To assess the *potential benefit* of the proposed project, members of the Federation of European Laboratory Animal Science Associations (FELASA) working group on ethics review (Smith et al. 2007) recommended that committees ask how original, timely, and realistic the objectives are; whether there is replication of previous work; and how the proposed work relates to other work in the field. To assess the *likelihood of achieving the potential benefits*, committees were recommended to consider the choice of animal model and scientific approach, the competence of the researchers, the appropriateness and quality of the facilities, and the way the results will be communicated.

In academic research, questions about how original, timely, and realistic the objectives are and how they relate to other work in the field are typically addressed in the scientific evaluation of funding applications. Therefore, it may be reasonable to suggest that the review committee for animal experiments should focus on addressing the likelihood that these benefits are achieved in practice. In animal research, the suitability of animal models is often a critical factor in determining whether or not the expected scientific and medical benefits are secured. In some areas of research, the choice of animal species to

be used is obvious—the agricultural scientist interested in aspects of dairy cow metabolism will develop his research on dairy cows. But in much fundamental biology and biomedical research, animals are used as models: researchers study animals of one species with the aim of gaining understanding with a wider application or with application to other species (typically humans).

Critical discussion of what characterizes a good animal model is curiously rare in the scientific literature. Most review papers on animal models limit themselves to an overview of the models and the connected discussion of any results that have been generated in studies using them. However, it has been forcefully pointed out that suitable animal models, and their appropriate use, are crucial in improving success rates in pharmaceutical development, i.e., in moving from a promising compound to an approved, marketable drug (Kola and Landis 2004).

The concept of *validity*, extensively developed in the field of neurobehavior following Paul Willner's (1984) enquiries into depression models, is useful in the critical evaluation of animal models. The most reliable measure of how well a model models is, of course, “predictive validity,” i.e., how well results obtained using the model predict outcomes in other species of interest. This can be assessed statistically, as has been demonstrated for type 2 diabetes research models (Varga et al. 2015). But it will often be many years before such information is available. In the development of treatments for human disorders, such validity is confirmed only when putatively effective compounds have made it all the way into studies with humans—a process normally taking at least 10 years. Therefore, researchers will seek earlier indications of model validity.

The notion of “construct validity,” recording how similar the underlying mechanisms of the model and the other species of interest are, is useful here. There is no quantitative method for ascertaining the construct validity of animal models. Instead, researchers must identify critical features of what is being studied (e.g., a disease mechanism in humans) and assess whether these are present in the animal model. For instance, limiting factors restricting the relevance of mouse models in tuberculosis research include the fact that the mouse is not a natural host for the bacteria that infects humans and that the most common strains of mice do not present the lung cavitation that is a key feature of disease transmission in humans (Fonseca et al. 2017). Unavoidably, scientists also operate under practical constraints. Most research is, to some extent, dependent on existing technologies. It is shaped by factors such as what models have been used before, what models the researcher has expertise in, whether an animal colony has already been set up at high cost, whether antibodies for the species are readily available, and so on. A telling example here

is provided by genetic models of Huntington disease. Heng and colleagues (2008) conclude that “the practical advantages of the strong R6/2 phenotype [with poorer construct validity] make it unlikely that it will be replaced as the preferred model. [...] The milder phenotype and late onset of behavioral abnormalities of transgenic full length and knock-in murine models [with better construct validity] make them difficult to use for preclinical pharmacology.”

This kind of decision reflects the reality in which scientists operate. Indeed, understanding a model’s limitations seems crucial in the discussion of validity. Garner and colleagues (2017) recently proposed the concept of *therioepistemology* as “the study of how knowledge is gained from animal research.” Central to this concept is the notion that all models are imperfect and that what matters is how the imperfections affect what can be gained from research employing a model. Therioepistemology, Garner and colleagues argue, should provide the framework to critically assess model choice, limitations, and consequences by asking the following six questions addressing what is being ignored when a certain approach is chosen for a certain purpose:

1. What features of model biology will be ignored?
2. What features of human biology will be ignored?
3. What features of the measures will be ignored?
4. What features of background methodology and husbandry will be ignored?
5. What animal well-being issues will be ignored?
6. What principles of experimental design and statistics will be ignored? (Garner et al. 2017)

We will now look at the way in which experimental planning and design can affect research benefits because this is an issue around which considerable and challenging evidence has accumulated over the last decade. Much of this knowledge derives from reviews of animal research underlying the development of treatments for stroke in humans. In this field, a number of compounds have shown neuroprotective effects in animal models, but very few have turned out to be effective in clinical trials with humans (van der Worp et al. 2005). This could be explained by the fact that animals are poor stroke models for the human condition and offer low predictive validity. However, this is not the only plausible explanation. Researchers concerned over the limited translation of preclinical research results into effective human stroke treatments have carried out several systematic reviews of the earlier animal experiments and found a number of critical shortcomings in experimental planning and design.

In many of the animal experiments, for example, the efficacy of the prospective treatment was probably overestimated as a result of design bias. Animals were not randomly allocated to treatments, or researchers were not

blinded when they administered treatments (drug or control) or assessed outcomes. As a consequence, the odds of positive treatment effects were higher than they would have been had such measures been in place. Significant clinical differences were also an unwelcome factor, in that the animals used were generally young and healthy before the experimentally induced stroke, while human stroke patients are often elderly and hypertensive (van der Worp et al. 2010).

In the vocabulary of validity, we are talking here about issues of *internal* and *external* validity [see Garner et al. (2017) and Würbel (2017) for more detail]. Briefly, internal validity has to do with whether the observed differences between treatment groups are indeed due to the treatment and not some other factor that the researchers are unaware of—in this case, unconscious bias among personnel involved in the experiment. External validity refers to whether the results will also apply in a context different from that of the study—in this case, whether the results in the young, healthy experimental animals would also apply to elderly and hypertensive patients. Proper consideration of validity (and the lack thereof) has fundamental effects: when the preclinical mouse studies of drugs with promising results for the neurological condition amyotrophic lateral sclerosis (ALS) were retested with adequate experimental design, they were all found to have no effect (Perrin 2014).

A third issue that matters is communication. By and large, if research is to be beneficial, it will be important that its results are made public. Publication in peer-reviewed journals is a prominent feature of modern academic life, particularly in the sciences, and as is well known, the performance of today’s researchers is measured largely on the basis of the number of publications they have in influential journals. Thus, at least in academia, there is no doubt that researchers will invest time and effort in the communication of their results.

However, it is generally difficult to get studies with negative results (no effect of treatment) published. As a direct consequence of this, publications are likely to reflect only a subset of the research that has been carried out in the field. In a field with extensive data from many studies, it is possible to use statistical methods to estimate the loss of information between study completion and publication. For studies of ischemic stroke, Sena and colleagues estimated that 14% of studies that were completed remained unpublished, often because the results were negative. This leads to the overrepresentation of positive studies, which has consequences for analyses of the entire body of work, so-called meta-analyses. If studies with a positive treatment effect are overrepresented in the available data, the meta-analysis will generate an overestimate of the treatment effect—in the case of ischemic stroke, this was found to be by about one-third (Sena et al. 2010).

Unpublished negative results have additional ethical consequences:

the “publication bias” of journals in favor of hypothesis-confirming results [...] might be a reason for the slow progress in the development of new animal models and their validation. Negative results often go unpublished, and poor concepts, hypotheses, and models survive, notwithstanding a vast amount of contradictory data, merely because these data are not made available to the scientific community [...]. Publication of negative findings from well-conceived and performed studies can help investigators to evaluate and ultimately abandon the development of an invalid and irrelevant animal model and help reduce the unnecessary use of laboratory animals.

(van der Staay 2006, p. 147)

Let us briefly reflect on the question of what is included in the definition of benefit. So far, we have focused on scientific and technical aspects of benefits. These are probably the most important aspects from the perspective of those performing studies with animals, because they can be addressed and improved in the planning of experiments. But the studies of public opinion referred to earlier in the chapter did not consider scientific or technical aspects of the benefit a study may secure—the benefit aspect of research was based on *purpose*. We will come back to the implications of different approaches to defining benefit later in the section on harm–benefit weighing.

How Can Harm Be Minimized?

At the beginning of this section, we pointed out benefits and harms as key determinants of ethically acceptable research. The very reason there are ethical concerns about the use of animals in research is that such research may inflict harm. Concerns about compromised animal welfare are rarely addressed effectively via a demonstration of human benefit alone. Efforts to reduce the harm done to animals during the research are generally important as well. Indeed, in ethical terms harm reduction may be the more urgent concern.

The 3Rs principle of replacement, reduction, and refinement was introduced by Russell and Burch (1959) as a way to ensure that biological and biomedical research is performed with minimal harm to animals. This approach, which has gained wide acceptance internationally, seeks to reduce harm through three means: the replacement of animal research with alternative animal-free methods, the reduction of the number of animals used, and the refinement of methods to minimize the distress caused to any animals that continue to be used in research. In what follows, we shall examine each R in turn, seeking to emphasize the main consequences for animal research ethics.

The replacement principle enjoys an especially high standing among the 3Rs. It was the first of the Rs to be

introduced by Russell and Burch (1959), reflecting the order in which the authors intended the Rs to be considered. Questions about reduction and refinement are only relevant if replacement has first been considered and excluded. The goal of replacement also found widespread support, in part because it is the only goal that is fully compatible with the animal rights perspective, i.e., the view that animal use solely for human benefit should not be permitted.

In this sense, replacement is probably the easiest of the 3Rs to communicate: “not tested on animals” is a more powerful message than “tested on fewer animals” or “tested on animals whose distress was reduced.” Replacement models are also quite likely to employ technical and scientific innovations, typically the result of years of development, which may add to their allure. Recently, however, scientists have started to become aware of the potential risk of overselling replacement. Policy and planning for biomedical research will vary depending on how realistic one perceives the option of full replacement. Representatives of the animal rights movement often argue that full replacement is imminent. In contrast, laboratory animal scientists highlight that animals are still being used in large numbers and that this use is likely to continue.

Scientists reading this chapter will be aware that experimental procedures that do not involve live animals, such as *in vitro* (e.g., cell lines), *ex vivo* (e.g., tissue culture), and *in silico* (e.g., bioinformatics) methods, are widely used in research laboratories already. In fact, these methods, and techniques that employ animal research subjects, often work in a complementary fashion, and it is therefore common for scientists to argue that they only use animals when replacement is not possible. Typically, when scientists apply for ethical approval for their proposed studies, they are asked to explain why they cannot be carried out without animals. Typically, the answer given is that the study requires the complexity of a complete living organism and therefore cannot be conducted *in vitro*. The potential to challenge that answer is rising as new methods are developed. Procedures involving animals may come to be replaced by novel *in vitro* and *in silico* methods, by carefully designed studies with human volunteers, or by innovative uses of existing patient data.

In biomedical research, new techniques are approaching, or even entering, a sphere of research activity traditionally dominated by animal models. The rapidly developing field of organoid biology is receiving enormous attention as this chapter is being written. Organoids are multicellular systems that recapitulate critical characteristics of actual organs. Compared with traditional cultures of cells of a single type, organoid models are able to mimic functions and dysfunctions at the organ level. Therefore, they offer attractive model systems for research, both in more

fundamental studies into development, homeostasis, and regeneration and in applications in disease modeling, drug discovery, and personalized medicine (Rossi et al. 2018).

Innovative use of human data permitting animal replacement can be illustrated with two cases. Conventionally, the genetic effects, and effects of the intrauterine environment, on fetal origins of chronic disease are studied with embryo transfer or cross-fostering of animals. However, human medically assisted reproduction also results in parent–offspring pairs with contrasting combinations of genetic and environmental similarity/dissimilarity. Exploiting this, a research team has used fertility clinic records to build a database of information that can be used to study the effects of maternal-related prenatal environment directly on human data (Thapar et al. 2007). In a second case, a workshop bringing together scientists and advocates of alternatives to animal-based pain studies concluded that by listing studies using human volunteers, they could not only decrease the number of animals used but also produce more useful data that facilitated the establishment of direct links between the human subjective pain experience and the biological parameters under investigation (Langley et al. 2008).

While these examples concern research, the overall development of replacement methods has focused on routine testing, the production of biological material, and teaching. A steadily (albeit slowly) increasing number of alternative test methods have gained regulatory acceptance (Balls et al. 2018). Indeed, this development made it possible for the European Union to ban all use of animals in cosmetics testing in 2013 (Rogiers 2018). A number of teaching tools, ranging from videos, to interactive software, to highly sophisticated mannequins, allow living and euthanized animals to be replaced at various levels of teaching (Interniche 2019).

Let us turn to the reduction principle. In animal studies that involve harm, the use of fewer animals will normally cut (as it were) collective animal suffering: the primary ethical motivation for reduction. Reduction is also valuable for good resource management: laboratory animals, and their housing and care, are costly, and resources for research are limited.

However, reduction is probably the most controversial of the 3Rs. There is great political value in bringing down the numbers of animals used in experimental procedures as a whole, as the number of animals reported in annual statistics is a highly visible and easily understood aspect of research animal ethics. Much the same is true of replacement, as performing fewer animal-based experiments is also immediately recognizable in the statistics. However, the problem with reduction is that, as detailed analyses have repeatedly shown, in actual research the number of animals used in an individual experiment is often too small for the results to

be reliable. This, of course, has important implications for the validity of the research results. Within a larger review of methods in neuroscience, Button and colleagues (2013) examined the statistical power of animal experiments investigating sex differences in water maze and radial maze performance. The effect (i.e., how large a difference there is between male and female animals) was calculated through a meta-analysis, and the authors then established how many animals a single study would need to detect effects of this magnitude with different levels of statistical power. To achieve 80% power (a common standard), 134 animals would be needed for a water maze experiment and 68 for a radial maze experiment, whereas the average actual sample sizes were 22 and 24 animals, respectively. As was the case here, it is in many cases misguided to seek to achieve reduction by bringing down sample sizes in individual experiments.

Of course, one of the most powerful means of reducing animal numbers is by replacing animal use with non-animal methods. Combinations of strategic reduction initiatives can also have a significant impact. Törnqvist and colleagues reviewed reduction measures in a pharmaceutical company and found that

“substantial reduction in animal use was achieved by different strategies, including improved study design, method development and project coordination. Major animal savings were shown in both regulatory and investigative safety studies. If a similar (i.e., 53%) reduction had been achieved simultaneously within the twelve largest pharmaceutical companies, the equivalent reduction worldwide would be about 150,000 rats annually” (Törnqvist et al. 2014).

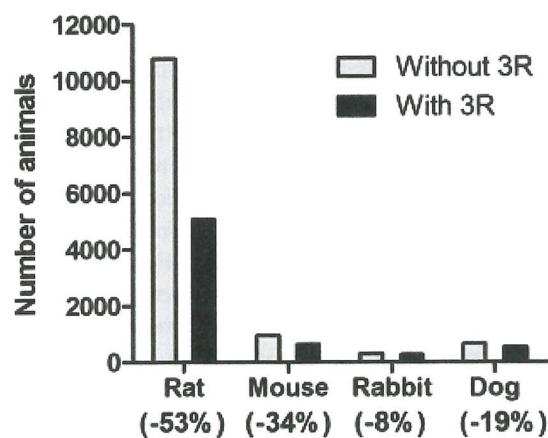


Figure 1.3 This graph shows the decrease in animal numbers achieved through different specific reduction projects in a pharmaceutical company. The dark bars show the actual animal numbers, and the light bars the estimated numbers that would have been used if the reduction projects had not been in place. (From Törnqvist et al. 2014.)

Once it has been shown that a research aim cannot be achieved without animal use, and once the animal numbers have been cut as much as possible, the refinement principle urges us to minimize any pain or distress that will be caused by adjusting the experimental procedures. Few people would challenge this principle, at least so long as conformity with it poses no threat to scientific results and does not require exorbitant funding.

Experiments can be refined in various ways, and we will mention only a few. The most obvious and direct strategy is to adapt the experimental procedures so that they cause less pain or distress. But in addition to this, the housing and day-to-day care of experimental animals can often be improved. Environmental enrichment, i.e., the provision of resources that enable animals to interact with, and control, features of their environment and to engage in motivated behaviors, normally improves animal well-being (e.g., Bailoo et al. 2018). The way that mice are handled (picked up) has a considerable effect on their anxiety, with tail-handling being much more aversive than open hand or tunnel-handling (Hurst and West 2010). In many invasive procedures, appropriate anesthesia and analgesia can play a vital role in pain management (see Hedenqvist, this volume). Where animals are experimentally required to develop a progressively severe condition, as happens in degenerative disease models, an important refinement can be achieved by carefully specified humane endpoints. In this scenario, the technician or researcher uses clinical signs as endpoint parameters, rather than awaiting the animal's spontaneous death (see Morton and Hau, this volume).

Interestingly, refinement measures are often scientifically relevant. Environmental enrichment can be employed to create systematic variation, thereby improving external validity (Bayne and Würbel 2014). Tail-handled mice show less exploratory behavior than mice handled using nonaversive methods, which in turn affects their performance in many behavioral tests (Gouveia and Hurst 2017). Severely affected animals not offered the refinements of housing adaptation and humane endpoints are likely to die from secondary causes (e.g., dehydration or malnutrition in rodents unable to feed and drink from the cage top), rather than the disease being studied (Franco et al. 2012). Survival/mortality when the cause of death is unknown or only indirectly related to the disease is not a high-quality variable to measure. As demonstrated by Scott and colleagues (2008), failure to consider nondisease-related mortality in a neurodegenerative disease model may even account for false treatment effects.

The three principles involved in the 3Rs do not operate in isolation and may sometimes conflict. Perhaps the most familiar conflict is that between reduction and refinement. This can arise relatively easily because lowering the total number of animals used will sometimes place a greater burden on each animal that continues to

be used. Examples include the reuse of animals in different experiments versus the use of naïve animals for each experiment; taking more blood from fewer animals versus a smaller amount from a greater number; and, in toxicology, testing the use of a higher dose (which produces a greater effect and thus requires fewer animals but may cause more serious harm to each animal used) versus using lower doses on more animals (de Boo et al. 2005).

Some people take the goal of reduction to be based on reverence for life (i.e., the notion that one should, as far as possible, avoid taking the lives of animals), but in other human uses of animals, including food production, this argument seems to have little public support. Instead, the prevailing ethical reasoning is something like: "It is OK to kill animals as long as they have good lives while they are alive." Extending this line of thinking, one could argue that killing more animals is acceptable if it allows each animal that is used to live a better life, particularly in situations where a focus on keeping the numbers down results in living conditions in which the animal is considered unfortunate to be alive (Sandøe et al. 2015). Weighing animal numbers against the burden on individual animals in this way could also be supported by a moral view that gives weight to "fairness to the individual animal" (Tannenbaum 1999), i.e., by spreading the load of suffering.

The way in which people balance the harm of killing against the harm of suffering will vary according to how much value they place on each harm. When participants in laboratory animal science training courses were presented with a hypothetical choice between exposing the same mouse to 20 procedures or 20 mice to one procedure each, 40% considered doing greater harm to fewer animals the ethically preferable alternative, while 60% felt it was more ethical to use more animals while reducing the harm done to each individual (Franco and Olsson 2014).

Also, the notion of "animal numbers" is less clear than might initially be supposed. What are we to reduce? The total number of animals used? Or the number of animals used relative to scientific output? After a period of steady decline, laboratory animal use figures have risen over the last few years (Speaking of Research 2019). However, increased investment in biomedical research may mean that the number of animals now being used relative to the amount of scientific activity taking place is actually falling.

Challenging the Status Quo

The requirements presented thus far are compatible with the moral framework of animal welfarism. According to this framework, it is always acceptable to use animals for research aimed at providing human benefits, as long as the animals are used in a way that makes it most likely the benefits will be achieved, and provided that the animals

are not harmed any more than necessary relative to the goal. However, recently, additional requirements have been proposed and implemented in some areas that go further than this. We will look at three of these requirements: harm–benefit analysis (HBA), banning or limiting the use of certain species, and a cap on animal suffering.

Harm–Benefit Analysis

In the section on how to maximize benefit, we addressed the benefit of animal experimentation from a technical perspective. It is obvious that a poorly designed experiment that cannot give a reliable answer to the questions it poses will not be beneficial; it may indeed even be harmful if it produces misleading results. But technical quality is only one of many aspects of benefit. The question of what it means for an experiment to be beneficial opens up a complex discussion about the various notions of benefit outlined earlier. That discussion becomes even more complex when we move from estimating benefit to weighing this estimated benefit against predicted animal harm.

There has traditionally been some reluctance within ethics committees to take on this discussion, sometimes even in performing the benefit evaluation and attempting a relative weighing. However, increasingly it is required that, in addition to harm being minimized and benefit maximized, the two must be weighed relative to one another, with ethical acceptability determined by the balance between harm and benefit. From this view, research should only be allowed if the expected benefits of the research (the outcome in terms of knowledge, improved treatment options, etc., that can reasonably be expected to be generated) are proportional to the predicted harm to the animals.

The need for HBA of some kind was recognized when the first animal ethics committees were established by Swedish legislation in 1979, and this kind of calculation formed part of the assessment regime introduced by the 1986 Animals (Scientific Procedures) Act in the UK. The concept of HBA is now more or less embedded globally and is an explicit requirement in a series of regulatory documents across the world, including EU legislation; AAALAC International requirements; and the *Guide for the Care and Use of Laboratory Animals*, which regulates publicly funded research in the United States (Grimm et al. 2019).

Traditionally, HBA has involved reflection and discussion of the merits of the project, taking into account the harm caused to the animals. However, over recent years, a number of authors have proposed specific methodologies. The idea of a more systematic approach was originally developed in a seminal paper in which Patrick Bateson argued that harms imposed on animals must be proportional to the scientific value of the experiment. Bateson (1986) introduced the decision cube as a way to work out

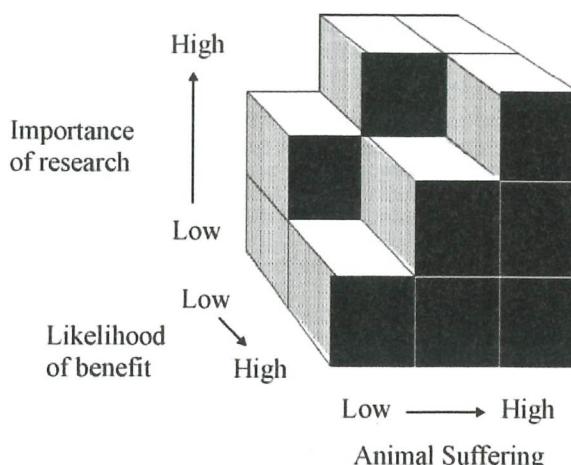


Figure 1.4 The Bateson cube for assessment of animal experiments. (From Bateson, P., *Advances in the Study of Behavior*, 35:211–233, Copyright © 1986 by Elsevier. Reprinted with the permission of Elsevier and the Bateson family.)

the acceptable level of animal suffering in an experiment relative to the value it has to humans, where the latter is defined in the two dimensions of “importance of research” and “likelihood of benefit.”

The exact ways in which to assess harm and benefit and weigh the two is less than straightforward. This is an active area where scholarly work, policy-making, and the critical assessment of policies and practice are continually moving the field forward. An important point on which opinions diverge is how broadly benefit should be defined. An expert working group with North American and European laboratory animal scientists has proposed that benefits should be defined in a set of distinct domains: social benefits (human health, animal health, and environmental health), socioeconomic benefits, scientific benefits, educational benefits, and safety and efficacy. In the assessment, benefit is further modulated by a set of factors: importance of outcome, clarity of objectives, translational potential, likelihood of success, continuity of recognized scientific efforts, quality of experimental design, innovation level, and dissemination results (Laber et al. 2016).

In their effort to develop a systematic approach, the working group brought together components from a number of previous approaches, resulting in a very broad framework. Such a broad definition is not universally approved. Several recent papers, based primarily on the extensive critical analysis of research methodology that we refer to in the section on how to maximize benefit, propose methods for *benefit* assessment exclusively based on scientific considerations. Briefly, they propose a parallel to the 3Rs as an approach to benefit assessment: the 3Vs. V here stands for validity: construct, internal, and external (Sena and Currie 2019; Würbel 2017).

Table 1.1 The Five Freedoms Can Be Used as a Measure of Good Animal Welfare, Where Harm to Animals Can Be Seen as Deviations from the Freedoms

1. **Freedom from hunger or thirst** by ready access to fresh water and a diet to maintain full health and vigor
2. **Freedom from discomfort** by providing an appropriate environment, including shelter and a comfortable resting area
3. **Freedom from pain, injury, or disease** by prevention or rapid diagnosis and treatment
4. **Freedom to express normal behavior** by providing sufficient space, proper facilities, and company of the animal's own kind
5. **Freedom from fear and distress** by ensuring conditions and treatment that avoid mental suffering

Source: Farm Animal Welfare Council (2009).

There is greater consensus over the definition and assessment of *harm*, where a large body of research in animal welfare science can be drawn upon. The transatlantic working group on harm–benefit analysis defines harm in relation to the Five Freedoms, a 40-year-old framework originally proposed in the context of farm animal welfare regulation (Farm Animal Welfare Council 2009). Thus, the assessment process should consider to what extent the experimentation will affect animals' freedom (Table 1.1) from (1) hunger/thirst, (2) discomfort, (3) pain/injury, (4) their ability to express normal behavior, and (5) fear/distress. To this assessment is then added a layer of modulating factors related to the animals, their environment, and the experiment. Animal-related modulating factors include animal species, the number of animals involved, whether they are suited to the environment, and their health status. Experimental modulating factors include the intensity and duration of the harm inflicted by the experiment, the cumulative experience of the animals, the application of endpoints to curtail suffering, the possibility of complications, and phenotypic manipulations. Environmental modulating factors include housing, husbandry, and the competence of personnel (Laber et al. 2016).

HBA involves three steps: identifying the benefits and efforts to maximize them, identifying the harms and efforts to minimize them, and weighing total expected harm against total expected benefit in order to determine acceptability. The real challenge in HBA comes to the fore in the last step, the weighing. The main disagreement here is over what to include in the definition of benefit. We have seen earlier that some approaches favor a broad definition, whereas others focus on scientific aspects. On the surface, a broader definition of research purposes that is closer to delivering concrete benefit seems better aligned with the public perception of what affects the ethical acceptability of animal use in research. As we saw in an earlier section, research purpose matters, and it seems that more highly valued research purposes justify a greater degree of animal harm in the view of the public. However, broad definitions may still be problematic. As pointed out by

Eggel and Grimm (2018), assessment schemes based on a broad definition of benefit will attribute a higher benefit value to applied research, where a tangible benefit is more likely, than they do to basic research. However, it is unclear whether higher benefit scores for applied research are really what the policy makers had in mind. For example, the European Commission expert working group on ethics review and harm–benefit analysis saw the need to make “an acknowledgment that without basic/fundamental research, many of the subsequent applied benefits would not have occurred” (Anonymous 2013).

It is also unclear whether, in practice, this is how HBAs are carried out. If experiments with more concrete or highly valuable benefits justify higher levels of harm, one would expect this to be directly reflected in the percentage of studies classified as severe across different types of animal experimentation. But a recent review of HBA in the UK regulatory system reports the proportion of animals in severe studies in 2016 to be 2.1% for basic research, 2.9% for translational/applied research, and 15.8% for regulatory testing (Davies et al. 2017). In other words, there is a negligible difference between basic and applied research in regard to the acceptance of severe experiments, and severe experiments are much more common in regulatory testing. It is likely that the acceptance of severe experiments in regulatory testing simply reflects the fact that they are legally required there. The alternative explanation—that regulatory testing is perceived as an activity generating more valuable benefits than research, even applied and translational research—is less plausible.

Focusing the benefit assessment on the ethics review of studies with animals on scientific and technical aspects does not mean that broader aspects should be disregarded in the overall discussion of how animals are used in experiments. As argued by Eggel and Grimm (2018), the prospective review of animal experiments for authorization is probably not the correct place to evaluate the societal relevance of animal research. Instead, they propose that this should be done in an evaluation on a political level, based on retrospective systematic reviews.

This section has focused on harm–benefit assessment as part of prospective review before experiments with animals receive regulatory authorization and can therefore begin. However, based on the UK review referred to previously, Davies (2018) argues that the HBA should be viewed as an open, iterative process that will go on for as long as the project is running, or even longer, rather than a one-off assessment prior to the project's commencement. This view is also reflected in the requirement for retrospective review in European legislation. At the time of a retrospective analysis, the harms are known, a more realistic estimate can be made of the benefits, and the outcome of the HBA may well differ from that of the prospective review (Pound and Nicol 2018).

One subject that is sometimes linked to the idea of harm–benefit assessment is choice of species to be used in an experiment. The driver here is the idea that harms may be seen as more serious if animals of so-called higher species are used. We prefer to treat this as a separate issue in the next section.

Taking Animal Species into Account

Animals of very different species are used in research. The choice of animal is often dictated by the kind of research being done, of course, but it is also affected by additional factors: the experience and expertise of the researcher, the institution's facilities, legislation, and sometimes public discussion in the country where the work is being carried out. The significance of these additional factors is brought out by the fact that even in, for example, *in vivo* research in the neurosciences, where an animal with a complex enough nervous system to embody mechanisms for learning and memory formation is needed, the available research species range from nematodes to chimpanzees.

Does the choice of species matter when it comes to ethical evaluation of animal-based research? It does, and two concepts are especially important in this regard: that of sentience and that of the socio-zoological scale. We shall therefore organize what we have to say around these concepts.

One of the reasons why animal species may matter ethically has to do with sentience, the capacity to perceive or feel things. A sentient creature experiences the world around it (Duncan 2006). It may also experience feelings and emotions. In the words of Thomas Nagel in his classical analysis of this type of conscious experience, “the fact that an organism has conscious experience *at all* means, basically, that there is something it is like to *be* that organism” (Nagel 1974). We ourselves are beings of a kind for which “there is something it is like to be that organism,” and it definitely matters to us what the quality of that experience is, whether “what it is like” is pleasant or painful. In the same way, we assume, it must matter to a lion, or a rat, or a pig what their experiences are like. For several decades, this reasoning has been fundamental for animal welfare scientists.

Sentience is relevant to the choice of animal species because it is related to the complexity of an animal's nervous system. It is important to note, however, that this is an area where scientific understanding is as yet limited: neurobiologists have not yet managed to explain sentience in terms of the material mechanisms of the nervous system. In general, our belief that other individuals are sentient is based on the observation that they are behaviorally, anatomically, and physiologically similar to us. In other words, if an individual acts in a way that is similar to the way we would act in a certain circumstance, and if that

individual possesses something like a central nervous system, we regard it as probable, on the basis of an argument from analogy, that this individual is sentient. This reasoning is relatively uncontroversial for adult human beings, but when we extend it to nonhuman animals, the issues become more complicated. Here, verbal evidence is unavailable and the obvious similarities are more limited. Although common sense detects sentience in many species, the scientific case for attributing it obviously needs to be based on systematic evidence.

Smith and Boyd (1991) once set out a systematic method of assessment of the kind needed here. They provided a checklist of neuroanatomical/physiological and behavioral criteria that can be used to determine whether a nonhuman animal has the capacity for pain, stress, and anxiety. In relation to any of these kinds of experience, the checklist will include the possession of higher brain centers and evidence of behavioral reaction to potentially nociceptive, anxiogenic, or stressful experiences. Further evidence will accumulate if the behavioral reactions are modulated by drugs with a known anxiolytic or analgesic effect in humans. Evidence will also accrue if peripheral nervous structures (including receptors, signal substances, and hormones) are involved in each type of reaction, especially if there is a connection between these latter structures and the higher brain centers. As an increasing number of these criteria are met, the case for categorizing the animal as sentient builds.

When we look at the way taxonomically distinct animals fare under systematic scrutiny of this kind, we see that there are two important lessons to be learned. The first is that all vertebrates meet the criteria for sentience. When Smith and Boyd's original analysis was published, positive evidence existed only for mammals and birds, but it has now been demonstrated that the criteria are also met by fish [see Sneddon (2011) for an overview]. The second lesson is that the frontier is moving, but for many of the invertebrates, we still know too little to be able to say whether sentience can safely be attributed. Since the previous edition of this chapter, more attention has been paid to the possibility of sentience in insects (Barron and Klein 2016) and to measures to improve welfare in gastropod and cephalopod mollusks based on the assumption that they are indeed sentient (Winlow et al. 2018).

Ethically speaking, then, how important is sentience as a factor in the selection of species for animal research? In one way, it might be said to be very significant, in that it constitutes the basis for one important aspect of ethical concern: animal welfare. In another way, however, the significance of sentience as a criterion of species selection can be seriously doubted. It is relatively poorly understood, and it is attributed to animals largely on the basis of imperfect analogies. Worse, a systematic checklist of scientifically respectable indicators shows that sentience is possessed by all vertebrates, and possibly some

invertebrates. That is not of much help if one is trying to determine which species to use in a potentially painful *in vivo* procedure.

There is a rather different way to approach the question of how animal species matters. Throughout human culture there is clearly a perception of hierarchy among animals, a quasi-moral ordering that gives some species higher status than others. This hierarchy has been labeled “the socio-zoological scale” (Arluke and Sanders 1996). The central idea of the scale is that people rate animal species as morally more or less important, and therefore more or less worth protecting, on the basis of a number of factors. These include how useful an animal is, how closely people typically associate with it, and how “cute” it is. They also include how dangerous the animal is capable of being and how “demonic” it is perceived to be.

Clearly, the socio-zoological scale varies from place to place and time to time, but today at least, and in western societies, some companion animal species, notably dogs and cats, seem to be at the top of it. Among other animals, large carnivores and nonhuman primates also are placed at the top end of the scale. In the middle are large farm animal species, such as cattle and pigs. Toward the bottom are pests or vermin, such as rats and mice. Fish, viewed by some to be alien, cold, and slimy, also appear to be quite low down the scale. So, among the animals potentially available for use in research there is a hierarchy, running from primates at the top, to rodents and fish, and on down to insects and other invertebrates.

The socio-zoological scale is, in many ways, based on tradition and unexamined prejudice, and its use as a basis of animal protection can be criticized both scientifically and ethically, but it undoubtedly plays a role in society. During the first trimester of 2019, the U.S. Congress introduced two bills specifically targeting research with dogs and cats, effectively closing a U.S. Department of Agriculture (USDA) research program with cats (Gregorian 2019). On the other hand, the vast majority of research animals that are used (rats and mice bred specifically for research) remain explicitly excluded from protection by federal legislation in the United States (AVMA 2019).

Stronger protection for species of greater public sensitivity is common. In EU legislation there is a restriction on the use of nonhuman primates. On the surface, the restriction seems strong, as the legislation states such primates “shall not be used in procedures.” However, exemption clauses moderate this, so that for nonhuman primates that are not great apes, the only limitation, in effect, is that use of these animals is only acceptable for certain research purposes. The strongest restriction is on great apes, but even they are not protected in all circumstances, since the legislation incorporates a general clause that offers provisional permission for otherwise banned experiments if they are essential “for the preservation of the species or in

relation to an unexpected outbreak of a life-threatening or debilitating clinical condition in human beings” (Olsson et al. 2017).

There is also growing public resistance to continued use of nonhuman primates. Airlines transporting laboratory animals have been the target of campaigns by nongovernmental organizations (NGOs), and many major airlines no longer transport primates for research (Wadman 2012). European scientists working with primates report that they are moving their research to Asia as a result of the difficulty, confirmed or anticipated, of getting such research approved in European countries (Abbott 2014).

Putting a Cap on Animal Suffering

A third requirement in the move toward a new standard for animal research ethics is to put an absolute cap on the level of suffering that animals can be forced to endure as part of an experiment. According to this requirement, experiments should *never* be allowed if they involve *severe suffering*. Such a requirement has been in place in Danish legislation for more than two decades. It is also included in the recent European Directive 2010/63/EU, which defines minimum standards for the regulation of animal experimentation put in place in each of the 28 EU countries. Thus, the directive [Article 15(2)] states explicitly that “Member States shall ensure that a procedure is not performed if it involves severe pain, suffering or distress that is likely to be long-lasting and cannot be alleviated” (European Union 2010). However, an important proviso is introduced by a further clause allowing the ban to be lifted “for exceptional and scientifically justifiable reasons” [Article 55(3)].

Such a ban on animal experiments involving severe suffering seems to move away from the harm–benefit approach in that it bans certain experiments, and thus protects animals, without considering the potential benefits of the experiments outlawed. Also, unlike the general requirement for refinement, which is always relative to what is possible without sacrificing the goal of the research, this requirement is absolute.

The concept of adaptive behavior is critical to our understanding of the qualitative difference between severe and less severe suffering. With less severe suffering, a human or animal will show allostasis (Korte et al. 2007). An adaptive response is mounted, and stability can be regained after the physiologically or psychologically stressful events cease. Events that result in severe suffering, on the other hand, are destabilizing, and physiological or psychological stability cannot be regained even when the external situation improves. Severe suffering is thus associated with a failure to cope (such that the current trajectory will lead to premature death) or with a long-term struggle to cope, in which all individual

biological resources have to be devoted to counter the situation. In such cases, the individual is fundamentally changed for the worse.

Combining literature from the human psychology of suffering, where self-reporting is possible, with insights from animal welfare science, in a recent paper (Olsson et al., *in press*), we have attempted to explain why severe suffering is more than a mere quantitative increase in negative state. Severe suffering involves a qualitative shift to a situation (not necessarily exclusive or complete) in which (1) suffering dominates attention; (2) there is limited capacity for distraction or compensation; (3) normal life cannot be pursued; (4) full recovery cannot occur, even if the external situation improves; and (5) in humans, at least, one's own life is judged not to be worth living. We argue that the avoidance of severe suffering clearly matters from many different ethical perspectives, including the view that animal harms can be balanced against, and offset by, human benefits. Here a ban can be justified in roughly the following way. If scientists are allowed to do experiments causing severe animal suffering, they will find a justification to do that. If scientists are not allowed to do experiments with severe suffering, they are likely to find an alternative way to achieve their aims. And indeed, in most (if not all) cases, it is possible to avoid imposing severe suffering on animals during experiments without giving up the potential benefits of new ways to cure, prevent, or alleviate serious human diseases. The example of measuring survival is illustrative. Originally, this was measured literally by whether animals died or survived. With the introduction of the concept of "humane endpoints," animals who were very severely affected, essentially moribund, were euthanized rather than awaiting spontaneous death. But as our understanding of disease advances from the organismic to the microscopic and molecular scales, it is now possible to adapt so-called "surrogate endpoints," based on biomarkers, rather than on overall clinical status. Such endpoints as alternatives to survival have been adopted as approval criteria for many human clinical trials. Regardless of whether there are candidate or regulatory surrogate endpoints or clinical biomarkers, the severity of the suffering imposed in many animal models can be mitigated by providing supportive care to the animals, without jeopardizing the scientific aims of the protocol, as already discussed in the section covering the refinement principle.

MECHANISMS FOR SETTING AND MAINTAINING STANDARDS

In previous sections of the chapter, we have tried to describe and explain the theoretical basis of a range of ethical norms and standards applying to laboratory animal use. In this final section, we turn to look at how these standards are maintained. The maintenance of standards

in society is invariably achieved through a combination of "hard" regulation and "soft" promotion. People are encouraged to act in ways society deems acceptable both by rules (some of which are backed by sanctions) and by policies that promote a positive attitude to the values underlying those rules.

Though we focus mainly on regulation here, we do not underestimate the importance of soft promotion of responsible attitudes to animal research. Nobody imagines for a moment that sexism, for example, can be eradicated from society through legislation and regulations alone. We know that policies reinforcing sexist culture and values are also required. Similarly, it is impossible to ensure that animal-based research is ethical simply by imposing rules and regulations. Ultimately, the aim must be to create and maintain an animal research community identifying with the values that underpin the rules, a culture within animal experimentation of ethical responsibility, or what is often termed a "culture of care" (e.g., Klein and Bayne 2007).

With that important proviso, we turn now to regulation. Who is responsible for ensuring that animals are treated ethically in the laboratory and how well do they do their job? The first part of this question is much easier to answer than the second. Most animal-based research is funded, directly or indirectly, by public money. This means that the public, or society as a whole, must be counted among a research institution's stakeholders.

Society uses a number of mechanisms to guarantee that research on animals is carried out in an acceptable way. In some places, the main instrument for regulating animal research is legislation. This is the case in Europe, where especially in the European Union, policy-making on animal research takes place at an international level through a combination of supranational and intergovernmental processes. Codes and regulations published by public bodies, such as the Canadian Council for Animal Care or the U.S. National Research Council, are other mechanisms helping to ensure that animal experimentation is performed in a societally acceptable way. However, the process by which these regulations are developed and updated is almost always slower than the rapid development of science and technology, and they are, by necessity, drafted in broad terms in order not to promptly go out of date.

In most places, therefore, the real decision-making on the treatment of animals in research projects is usually delegated to an ethics or animal care and use committee. Committees can act more flexibly. They are also able to enter into dialogue with scientists proposing experimental projects, and in that way, challenge scientists to develop their research in line with evolving best practice. Ethics committees and other similar bodies are often the only formal regulatory bodies tasked with looking in detail at research projects, and they may therefore have a very

strong impact on the way experiments are designed and carried out in practice. In recognition of their important role, the workings of such review committees have been researched intensively over the last decade, and we now know that important aspects of how well the review process works include committee composition, committee dynamics, and the kind of information on which committees base their decisions.

A complete and transparent review process is dependent on committee composition and dynamics; it should represent all important stakeholders in the discussion equally. There seem to be at least three main stakeholders: researchers/industry, interested in being able to perform their proposed studies; animals, interested in being protected from harm; and society, interested in seeing that acceptable standards are maintained. The research and animal interests are generally represented in review committees, typically through the participation of scientists, who represent the research, and by veterinarians and animal care personnel, who represent the animals. There is greater variation in the way the societal perspective is represented. Often, this is through special interest representatives (most commonly, members of animal protection groups, who can also claim to represent the interests of animals), but a number of European countries do not include societal representation at all in the review process (Olsson et al. 2017).

The involvement of a wide range of parties with various approaches to the ethical issues raised by animal research will help reduce the risk of committees becoming biased, but inclusive committee composition may not be sufficient to guarantee a participatory process. A number of studies show that scientists tend to dominate discussion. Their expertise in science, which is both an important knowledge domain for the committee's discussion and generally difficult for the nonscientist to access, probably contributes to this imbalance. Committee dynamics may also be influenced by how the decision-making process is organized. Most committees operate according to what might be termed a discourse model and form opinions on research applications through dialogue among the members. However, in the absence of a clearly defined process for decision-making, as is common in the committees, deliberations risk focusing on issues that the most active members are most comfortable discussing, i.e., scientific and technical aspects [see Grimm et al. (2019) for a discussion of this]. More systematic approaches, such as the one proposed by the American Association for Laboratory Animal Science (AALAS)-FELASA working group on harm-benefit analysis (Laber et al. 2016), may help with this problem. However, the quality of committee decisions also depends on their members having access to full information. Vogt and colleagues (2016) found that research applications often failed to include important information about issues that are critical for scientific validity, such as

sample size calculations and measures against bias, leading them to argue that "authorization of animal experiments is based on confidence rather than evidence of scientific rigor."

Regulations and ethics committees are, at least to some extent, external ways of regulating animal research. But the scientific community is also increasingly developing internal mechanisms involving various kinds of guidelines and checklists. Following the development of a series of discipline-specific guidelines, especially in the neurosciences, the Animal Research: Reporting of In Vivo Experiments, or ARRIVE, guidelines were the first set of recommendations designed to apply to a wide range of research with animals (Kilkenny et al. 2010). They were developed by the UK National Centre for the 3Rs (NC3Rs) to improve the standard of reporting of research using animals. Together with their corresponding checklist assessing whether adequate information and detail have been included in submitted manuscripts, the ARRIVE guidelines have been widely endorsed by research institutions, funding agencies, and over 1,000 scientific journals.

However, despite this widespread support, there is still only limited evidence that the reporting standard has, in fact, improved, motivating NC3Rs to review the guidelines and develop a strategy to accelerate their uptake (du Sert et al. 2018). The laboratory animal science community has also recognized that improving reporting is not enough and that "there is very little overarching guidance on how to *plan* animal experiments, despite the fact that this is the logical place to start ensuring quality." This has led to the recent introduction of "the PREPARE guidelines: Planning Research and Experimental Procedures on Animals: Recommendations for Excellence. PREPARE covers the three broad areas which determine the quality of the preparation for animal studies: formulation, dialogue between scientists and the animal facility, and quality control of the various components in the study" (Smith et al. 2018).

Since publication of the previous edition of this book, the scientific community has also engaged with animal research reporting in ways we were hardly expecting 10 years ago. The first initiative toward greater openness about research with animals was the 2010 Basel Declaration (Abbott 2010), which was the stepping stone for an international organization aiming to "strengthen public awareness of the importance of animal models in experimental biomedical research, to foster communication between researchers and the public" and "to bring the scientific community together to further advance the implementation of ethical principles such as the 3Rs whenever animals are being used in research." In 2012, more than 40 research organizations in the UK signed a declaration that later led to the Concordat on Openness on Animal Research. This lists a set of commitments requiring research institutions to communicate more openly

about how they use animals in research (Anonymous n.d.). Originating in the UK, this initiative has spread to other European countries, and transparency agreements have now been signed in Spain and Portugal (EARA 2018). EU legislation is also promoting a degree of transparency about research through the requirement that so-called nontechnical summaries of approved research with animals must be made public (European Commission 2018).

CONCLUSION

Animal experimentation remains a controversial activity, and at the heart of that controversy is how to balance the concern for protecting animals from harm against ensuring research findings that may be of benefit to humans. Whereas this basic dilemma remains unchanged, many aspects of the context around animal use in experiments are changing. The societal view of what is acceptable seems to be evolving from one that accepts any animal experimentation, as long as it serves a useful purpose and harms animals only as much as necessary to achieve the goal of the experiment, to more restrictive views on animal use. This development is reflected in regulatory measures that are tightening the requirements on animal experiments, e.g., aiming to enforce a robust harm–benefit analysis and putting a cap on animal suffering.

Within the scientific community there is increasing awareness of the need to improve practice to meet these requirements, although this is probably still mainly at the level of critical discussion and analysis. And there is also a changing approach to communication, in which scientists and research institutions are shifting from a degree of secrecy about animal experimentation to a new commitment to openness and dialogue on the ways in which animals participate in experiments.

These are all welcome developments. They are likely to lead to better informed discussion and, indeed, improved practice in terms of the harm–benefit balance in research with animals. But public controversy is unlikely to disappear as a result. Animal experimentation will not be banned (or replaced by alternative methods) by 2021, as required by the initiative mentioned at the start of this chapter. More systematic and demanding approaches to harm–benefit analysis will not ensure that we all agree on what makes an experiment ethically acceptable, and institutions speaking openly about their research with animals will not make public criticism go away. As in all other domains in which animals play a role in human life, a troubling dilemma between the service of animal and human interests will persist, and there is no obvious way to settle that dilemma once and for all. The best that we can hope for is continued effort to ameliorate that dilemma and maintain open and honest dialogue about it.

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