Ethical Considerations in Mouse Experiments

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ABSTRACT

Mice count morally because they can be harmed. This raises a moral issue in animal experimentation. Three main ethical attitudes towards animals are reviewed here. The Kantian view denies moral value to animals because they lack reason. The second view, by Singer, considers animals as sentient creatures (i.e., able to suffer). Finally, Regan considers that animals are subjects of their own life; they are autonomous and therefore have moral rights. Singer is a reformist and allows animal experimentation under certain conditions. Regan is abolitionist, saying that animals have moral rights that cannot be negotiated. Current animal protection legislation strives to put in balance the human and animal interests to decide whether an animal experiment is morally justified or not. An ethical evaluation process is conducted based on the harm-benefit assessment of the experiment. The researcher has to implement the 3Rs (Replacement, Reduction, Refinement) to minimize the harms to the animals and make sure that the outcomes are scientifically significant and that the quality of the science is high, in order to maximize benefits to humans and animals. *Curr. Protoc. Mouse Biol.* 1:155-167 © 2011 by John Wiley & Sons, Inc.

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INTRODUCTION

Why should we introduce ethical considerations into experiments with mice? The fundamental reason is that humans interact with animals differently than with objects, plants, or microbes. For example, when I walk along a path in the wild, I often kick a stone inadvertently. I do not bother about it (except if the stone could hurt somebody). I may pick up a bunch of weed to clean my shoes. I do not bother about that, either. But if I step on a tiny frog and hurt it, I grieve. Why? Because frogs matter to humans, contrary to stones and grass. Frogs matter because they are living beings, and because we tend to think that harming an animal is something we should avoid whenever we can. In short, animals do morally count for us, and it is not permissible to behave with respect to them just as we choose.

Animals morally count because they can be harmed, but how much do they count? Human beings count, too, and sometimes, we meet with conflicts between animal and human interests. This is the case not only in animal experimentation: we eat farm animals; we destroy pests. Even with pets: can we say with confidence that we always act in their interests (think of castration)? Animals and human beings are related in many ways, and their interests interrelate in multifarious ways.

In this overview, we will examine the case of mouse experiments. Laboratory mice are used all over the world and are the most frequently used laboratory animals in many countries. The principal reason is that, thanks to the particular reproductive biology of this species, mouse genetics is easy to manipulate. We will put our inquiry in the larger context of animal experimentation and the requirements that animal ethics exert upon on it. We will briefly discuss whether genetic manipulations are ethically defensible or not.

Definitions of some of the philosophical terms used in this article are provided in a glossary in the appendix at the end of the text.

ANIMAL'S MORAL STATUS: SEVERAL THEORIES

Moral status, intrinsic and extrinsic properties, and properties and values

Stepping on a stone is not the same as stepping on a frog because we feel responsible for having "hurt" a living organism. In ethical terms, animals have a *moral status*. What is it,

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more precisely, to have a moral status? Mary Anne Warren states that: "To have moral status is to be morally considerable, or to have moral standing. It is to be an entity towards which moral agents have, or can have, moral obligations. If an entity has moral status, then we may not treat it in just any way we please" (Warren, 1997).

Animals deserve moral consideration; therefore, we have moral duties toward them. But why? Giving a general answer to this question is easy: the moral status of an entity depends on certain of its *intrinsic properties*. They are not to be found "outside" of the object. My dog, born on June 9, 2000 in La Sarraz, with name Bobby, blue eyes, and black and white fur, has intrinsic properties such that we may relate to its feelings and capacity to suffer; however, my dog has also extrinsic properties or relational ones because this particular dog matters to me, as I have spent many years with it, shared many treks in the mountains, etc. Warren (1997) characterizes both properties as follows: "A thing's intrinsic properties are those which it is logically possible for it to have had were it the only thing in existence. Its relational properties are those that it would be logically impossible for it to have had were it the only thing in existence." And, as some intrinsic properties confer intrinsic value, some relational ones confer relational value. For example, 'to be loved' is a relational property that confers relational value upon the entity that is loved (the object of love); 'to be hated' brings relational disvalue. As this example shows, relational value can combine with intrinsic value to enhance the general value of the entity, or diminish it if it is a negative value (a disvalue). As we will see, the very different attitudes that we adopt toward mice, considered as laboratory animals, pets, or pests, do not depend on their intrinsic properties (all mice have the same relevant moral intrinsic properties) but on their relational ones.

Intrinsic properties, as opposed to *extrinsic properties*, have to be found in the object itself. Going back to our first example about stones, weeds, and frogs, the latter is a living entity which is clearly different from a stone or other nonliving objects. A frog also has something more than weeds or other plants. What makes animals different from the rest of the world? Here philosophers and ethicists have different views of these properties; for Singer, the key property is sentience, for Regan, a kind of subjectivity (see below for more details).

Intrinsic properties like sentience or subjectivity are constitutive of moral status because they possess intrinsic value; beings who have moral status possess a value by and in themselves, and we ought to respect them because of this value. Regarding human beings, this intrinsic value is often named *dignity*. Aquinas said long ago: "Dignity means the goodness [or value] a thing possesses because of itself; utility, because of another thing" (*Scriptum super Sententiis*, liber 3, d. 35, q. 1, a. 4, q. 1, c. Available at: *http://www.corpusthomisticum.org/iopera.html*; accessed 7 May 2010). Moral status of an object takes roots in its intrinsic value that originates in its intrinsic properties.

The moral status of mice and other animals calls for moral duties towards them. Are these duties identical to the ones we have towards humans or are they different? If they were identical, most studies on mice could not be possible; most animal experimentation should be banned. How different then are duties towards animals and humans?

To answer this question, we have to go back to the relevant intrinsic properties of animals and humans. Those properties are identified on the basis of several predicates, such as those listed in Table 1 (Beauchamp and Walters, 1989). According to Pucetti, says LeRoy Walters, "the S*-predicates in the left-hand column can be applied to conscious nonpersons like dogs, whereas the R*-predicates in the right-hand column presuppose the possession of a conceptual scheme and the capacity to act as a moral agent. This latter capacity is, for Pucetti, the primary distinguishing feature of personhood, for persons are the only conscious entities who can adopt moral attitudes toward moral objects" (Beauchamp and Walters, 1989). In short, the moral status of these beings depends on what they intrinsically are, respectively, beings with properties sustaining S*—animals—and beings with properties sustaining R*—human beings.

In comparing human beings and animals, we have highlighted two sets of properties. Does it mean that there exist at least two different moral statuses?

Certain authors think like that, but others, strictly following the conception put forward by Warren, claim that there is only one moral status, the possession of which determines moral duties: "If an entity has moral status, then we may not treat it in just any way we please." If we consider the objects that have moral status, their moral status is the same.

Table 1 Morally Relevant Intrinsic Properties

S*-predicates/animals	R*-predicates/humans
To be in pain	To want to secure justice
To feel hungry	To summarize the point nicely
To be excited	To be an astute judge of character
To be afraid of you	To be a smug hypocrite

Traditionally only humans had been considered possessing moral status.

Indirect duties theories

Human beings, and only them, have moral status, because they possess the only intrinsic property that has moral relevance: *reason* (autonomy, self-consciousness, self-mastery,...). Kant is very clear on this point: "The fact that man is aware of an ego-concept raises him infinitely above all other creatures living on earth. Because of this, he is a person [...]. He is a being who, by reason of his pre-eminence and dignity, is wholly different from things, such as the irrational animals, which he can master and rule at will." (Kant, 1978; originally published 1798). Animals have no moral status because they are lacking in rationality; therefore they are things, that is, entities that can be possessed, sold or destroyed.

We "can master and rule [animals] at will" says Kant. Does it mean that we can treat them with violence and cruelty? Kant answers clearly no. However the reason he gives has nothing to do with a wrong committed against them. Animals have no moral status; therefore they do not belong to the moral community, which comprises the set of beings who have moral duties toward each other. Animals can be *harmed*; they cannot be *wronged*. The reasoning against violence and cruelty thus consists in a duty toward human beings themselves: if I harm an animal without a good reason, I behave myself in a wrongful manner, because I manifest a vice of character—to be violent or cruel is a breach to a duty toward oneself, the duty to be gentle and benevolent—and the consequence thereof is that I will probably be violent and cruel toward my fellow human beings. For this reason, Kant condemns experiments with animals when they are unnecessary *and* done only for the sake of knowledge (Kant, 1996; originally published 1797).

Such a conception was often embodied in the law before the 20th century. For example, in France, the Grammont Law (enacted in 1850) forbade bad treatment of animals, but only in a public place. The reason is not that the animal would be wronged, but that passersby could be shocked by the cruel deeds. In brief, for this conception, we do not have direct moral duties toward animals, but only *indirect* ones: animals must not be harmed because it would wrong human beings (the doer and the observer).

Direct duties theories

For indirect duties theories, the protection of animals is grounded on relational properties, i.e., the *effects* our deeds have on human beings. Nowadays, the great majority of our regulations are grounded on *direct* duty theory. It is forbidden to treat animals with violence and cruelty because such acts *wrong* their victims. Therefore, for those theories, animals possess a moral status; they have intrinsic properties asking for respect and protection.

Utilitarian position (Singer)

The first and most well-known author who has endorsed a direct duty theory is the philosopher Peter Singer. He followed Jeremy Bentham when he stated: "The question is not, can they reason? Nor can they *talk*? But, *can they suffer*?" (quoted in Singer, 1979). The property relevant for the possession of a moral status is not reason but *sentience*, i.e., the capacity for suffering and for happiness. Human beings can suffer or be happy, but they are not alone; animals also can. Therefore, animals belong to the moral community: they count morally because their happiness counts.

As we can see, Singer, following Bentham (who was following Rousseau on this topic), enlarges the moral community. The relevant characteristic is no longer *reason*, possessed only by human beings, but *sentience*, belonging to human beings *and* animals (probably not all animals, but at least vertebrates). Reason is no longer the central intrinsic property, but sentience. *Pathocentrism* has replaced *anthropocentrism*, as we often call those

conceptions. Singer even compares anthropocentrism to racism, and makes this comment: "Racists violate the principle of equality by giving greater weight to the interests of members of their own race when there is a clash between their interests and the interests of those of another race. [...]. Similarly those, I would call, "speciesists" give greater weight to the interests of member of their own species where there is a clash between their interests and the interests of those of other species." (Singer, 1979). Human beings tend to be speciesists, even when they put reason to the fore, because reason is a property that, on the earth and in their mind, only their own species possesses; in this sense anthropomorphism is *speciesism*.

If sentience is the relevant moral property, then the interests of all sentient beings have the same importance. Therefore, all animals—human and non-human—are equal. We must give an equal consideration to their interests—the suffering of a mouse has the same value as the suffering of a human being. Does it mean that a mouse has the same moral importance as a human being? Not necessarily: human beings could be built so that their capacity for suffering would be much higher than that of mice. But the reverse could be true, also! It is an empirical question.

For the moral point of view, as Singer is an utilitarian, he feels no necessity to enter into those questions: what counts once and for all is the *quantity* of happiness and of suffering in play. "How bad a pain is depends on how intense it is and how long it lasts, but pains of the same intensity and duration are equally bad, whether felt by humans or animals." (Singer, 1979). In the case of an experiment with mice, what matters is the suffering of the mice and the benefits for the human beings (and maybe for the mice). Every benefit and every harm or suffering must be put into the balance. If the balance is in favor of human beings, the experiment is allowed; otherwise, it is not (and is immoral if performed in spite of the result of the balancing).

To realize a thorough balance of interests is not an easy matter, and we obviously must have recourse to some approximations and evaluations (see "How to undertake the ethical review process," below). However, ethology and animal psychology continue to progress. As a matter of principle, it must be noted that the Singerian position, which is at the root of the animal liberation movement, does not forbid animal experimentation. It is not an *abolitionist* position, but a *reformist* one; animal

experimentation is permissible when the sum of suffering is less than the sum of benefits. This seems reasonable and militates strongly in favor of measures like the three Rs (see "Points that the researcher should consider for a harm-benefit assessment in mouse experiments," below). Singer's perspective has its drawbacks too, as it would authorize an experiment performed on unwilling human beings in favor of a greater human happiness. Answers to this drawback of the utilitarian theory exist, but this does not concern us, because our topic of interest is animal, not human experimentation.

Rights theory (Tom Regan)

Peter Singer's position is reformist; Tom Regan's one is abolitionist. He objects to the use of animals for the sake of human interests—animals ought not to be used as mere means for the benefit of human beings; they must not be *instrumentalized*. What are his arguments?

Regan is not a utilitarian; therefore, for him, it is not the capacity to suffer, i.e., sentience, that counts. For him it is *Autonomy*. "Animals, as individuals who retain their psychological identity over time, have a welfare that is not unrelated to their ability to act autonomously (i.e., as they prefer)" (Regan, 1984). Autonomy is a rational property, and traditionally it has been the basis for the ascription of *moral rights*. Do animals have moral rights? Regan claims that they have. But how can he justify this claim, since animals are deprived of rationality? To understand Regan's position, some distinctions are in order. If traditionally, and especially in Kant's thought, autonomy is a rational property (the property of being able to lead one's life and to make choices and decisions after having examined the various possibilities) at the root of the various liberties and rights, this kind of autonomy is not the only one. To be autonomous still means to have the capacity to realize one's preferences: "Individuals are autonomous if they have preferences and have the ability to initiate action with the view to satisfying them" (Regan, 1984). Animals (at least some of them, and in particular mice) do have this property; they are, in this sense, the subject of their lives (Regan, 1984).

Animals and human beings are subjects of their own lives; consequently, they possess moral rights. It is easy to understand the reason for that. If a being has preferences he wants to satisfy, if he has projects he wants to realize, if he has a welfare that he cares for, then all those endeavors must be protected, in the

sense that they must not be interfered with without good reason. Such a being has a moral right to pursue his endeavors, and human utility does not justify a violation of such rights. Of course, animals are not human beings; notably, they are not moral agents (they do not act from a moral point of view), but moral patients (they possess interests with a moral weight). However, since moral patients as well as moral agents are subjects of their lives, they possess rights. Therefore, "it is not an act of kindness to treat animal respectfully, it is an act of justice" (Regan, 1984), i.e., an act of respect for their rights.

As animals and human beings are on a par concerning their rights, we understand why Regan is an abolitionist. Since it is immoral to experiment on human beings for the sake of the other human beings without their consent, it is immoral to experiment on animals without their consent. Since they are unable to give their consent, animal experimentation is immoral (like experimentation on children or on mentally handicapped human beings).

More precisely, Regan spells three objections against animal experimentation.

1. The right to medical treatment is an acquired right that we have with regard to society or the medical profession. It does not authorize governments to subsidize research that violates the basic rights of animals, or entitle scientists to conduct such experiments (Regan, 1984).

2. "Risks are not morally transferable to those who do not voluntary choose to take them" (Regan, 1984). Life is risky, and it is the function of medical and scientific research work to minimize, even to cancel, those risks. To do so, they must perform experiments, a risky procedure for the subjects involved in them. If those subjects consent to run those risks, they can be morally accepted; if they do not consent (because they don't want to, or because they are unable to), then this consists in an unacceptable instrumentalization.

3. It is morally impermissible to utilize beings that have an intrinsic value (Regan says "inherent value") like mere resources for other beings (Regan, 1984). Animals, like human beings, have a moral status; therefore they have an intrinsic value that should be respected.

Hierarchical theory

There is one feature common to direct duties theories we have examined so far, and to indirect duties theories: their monism or "centrism." A conception of moral status is monistic when it considers that there exists only one

moral status—either you have a moral status or you have no moral status. Those theories differ with respect to the property that is relevant for the possession of such a status—*reason* (according to Kant and many authors in our western tradition), *sentience* (according to Singer and the utilitarians), or subject-of-a-life (according to Regan). This property sets a threshold: if you are above, you possess a moral status; if you are beneath, you don't possess any. Monism breeds 'centrism'—anthropocentrism (only beings endowed with reason, i.e., human beings, possess a moral status) or pathocentrism (only beings endowed with sentience, i.e., which can feel pleasure and suffer, possess a moral status).

There is, however, another way to consider moral status: pluralism. A conception of moral status is pluralistic when it considers that there exist several kinds of moral status, depending on different properties, e.g., reason for persons, sentience for animals, or life for plants. Pluralism does not breed "centrism" but "hierarchism." It places the different moral statuses on a scale, usually with human beings at the top, animals a little beneath, then plants, and, perhaps, microorganisms.

This conception is widespread in our societies. It is often refined with subhierarchies, especially in the realm of animal experimentation. It is better to use mice than chimpanzees, rats than primates, and so on, because animals that are placed higher up on the scale have more intrinsic value. But it is not only folk ethics. The Nuffield Council on Bioethics (1996), for example, claims that it is better to use a swine than a chimpanzee for xenotransplantations.

Some authors object that such a view is the remains of a pre-Darwinian conception (the great chain of beings, the *scala naturae*). But this is not necessarily the case: all depends on the manner in which you conceive the hierarchy. You do not have to have recourse to biological criteria; complexity or other capacities can be appealed to, especially mental ones that are gradual or scalar, e.g., (self-)consciousness.

What makes the difference between a pet mouse, a lab mouse, and a pest mouse?

A hierarchical conception explains many judgments that we pass on the use of animals in experiments, but not all. What makes the difference in consideration between a *pet mouse*, a *lab mouse*, and a *pest mouse*? Mice may be considered as almost a family member, as a subject in a medical study, or simply as a

pest to be destroyed. Although these mice are almost identical in cognitive and emotional performance, and although their capabilities to feel pain and to suffer are certainly not very different—i.e., their intrinsic relevant properties are the same—our relationships to them are however quite diverse, even contradictory. As we have said, our attitudes depend here not on their intrinsic properties, but on their relational ones. Differences in relationships bring with them different ethical considerations. But is it morally justified to assign different moral status to different mice based on their relationships with humans?

Following the different direct duty theories we have examined, we ought to say that it is not justified to assign different moral status to different mice (Mason and Littin, 2003; Meerburg et al., 2008). As they have the same morally relevant properties, they have the same moral status. Therefore, their interests must be considered accordingly. The fact that a mouse has a different relationship to us does not change anything with respect to its intrinsic value. Consequently, if we adopt severe norms for animal experimentation, we should accept severe ones for pest destruction. Of course, the consideration of all interests involved may shift the balance (if you are not an abolitionist). Pest mice threaten our interests; lab mice do not. This fact can easily justify a difference in treatment, but not a negligence of the interests of the mice.

An Ethical Step Further: Animal Dignity

The special case of the Swiss Constitution and the law for protection of animals

Like all countries, Switzerland has a law for the protection of animals (LPA). But in contrast to others, this law is in part based on an appeal to the animal's dignity—and even to the dignity of creation. In the Swiss Constitution, we read: "The Confederation shall legislate on the use of the reproductive and genetic material of animals, plants, and other organisms. In doing so, it shall take into account the dignity of creation and the security of man, animal and environment, and shall protect the genetic multiplicity of animal and vegetal species." (Swiss Constitution, art. 120).

Animal and human dignity

Traditionally, dignity has been a concept restricted to human beings. In the 20th century, it has been more and more used, in the aftermath of WWII and of the *Universal Declaration of Human Rights*. We read in its first article: "All human beings are born free and equal in dignity and rights. They are endowed with reason and conscience." But what does it mean exactly to respect human dignity? Mainly two things, usually expressed in two bans:

1. a ban on instrumentalization, i.e., on utilizing human beings as if they were mere objects;

2. a ban on degrading treatment or humiliation.

These two bans are on the forefront in internationally important texts (e.g., *Convention for the protection of human rights and dignity of the human being with regard to the application of biology and medicine* (Oviedo Convention), and *Universal Declaration on the Human Genome and Human Rights* proclaimed by UNESCO). These texts are meant to protect human beings against abuses by medicine and biology that reduce them to the status of objects for experiment, treating them as nonpersons (Beyleveld and Brownsword, 2002).

The ban on instrumentalization is the most widely voiced of the two bans, but it is in a sense less fundamental than the ban on degrading treatments, because instrumentalization is only one kind of degrading treatment—it degrades a human being to the status of a thing. Nevertheless, if the two bans are kept separate, it is because each points to a different paradigm: the paradigm of instrumentalization is slavery, whereas the paradigm of degrading treatment is torture. Historically, slavery and torture are perhaps the two main domains where human beings have been (and sometimes still are) treated totally disrespectfully.

Animals are not human beings; in particular, they are not "endowed with reason and conscience," two properties at the root of human dignity. Moreover, although intentional torture is condemned when carried out against animals, methods for killing mice as pests are not always soft, nor is instrumentalization. In a sense, animals are our slaves. Pet and lab mice are used uniquely for human goals: a paradigm of instrumentalization. What does it mean then to respect the dignity of animals?

For abolitionists, the answer is straightforward—to respect animal dignity is to give up animal experiments and to refrain from interfering with their lives. For those who adhere to other conceptions, the answer will depend on the intrinsic morally relevant property of animals. To *possess interest* (that can be satisfied or frustrated) is characteristic of sentient animals. It is

consequently not surprising that this property has been chosen as a ground for moral consideration, i.e., dignity. Therefore, it seems natural to claim that to respect animal dignity amounts to satisfying their interests. More precisely, as the question of respect of dignity arises when there exists a conflict of interests between human interests and animal interests, the Swiss Federal Ethics Committee on Non-Human Biotechnology (ECNH, 2001) has stated: "We disregard an animal's dignity if we fail to make the possibility of violation the subject of an evaluation of interests, i.e., if we give it no consideration and take it for granted that human interests take precedence" (ECNH, 2001). Human and animal interests must be put in a balance. If our decision follows the tilt of the balance, then we respect animals' dignity; if we go on with the experiment contrary to the tilt of the balance, we violate animals' dignity.

The recent Swiss LPA has confirmed this view. It defines dignity as the proper value of an animal and states that there is an infringement of the dignity of the animal when the burden on it cannot be justified by human dominant interests. Under "burden," we must understand pains or harms, anxiety or debasement, profound modifications of its phenotype or capacities, and excessive instrumentalization (LPA, art. 3).

Note that in some other readings of the Swiss law and especially in the application decree, we find also another view. Animal dignity would be per se violated by certain painful or anxiogenic interventions, without any reference to a weighing of interests. Under this second interpretation, it would be lawful and permissible to violate the dignity of animals when important human interests are at stake; under the first (classical) interpretation, the importance of these human interests would prevent the act from constituting such a violation, if the interests of the animal have been taken into account (see Krepper, 2010; Swiss Academies of Arts and Sciences, 2010). To follow the second interpretation will of course favor the belief that animal experimentation is morally—if not legally—dubious.

Does genetic manipulation of the species raise special issues?

The answer to this question depends on which stance we adopt. For some authors, genetic manipulation can easily be considered as a violation of dignity. If changes in phenotypic traits count as harms, and if such harms are on the same footing as pain, anxiety, or debasement, then phenotypic changes due to a genetic manipulation will constitute infringements of dignity. On the contrary, if we adopt the Swiss conception of animal dignity, genetic manipulations will count only if they are against the satisfaction of an animal's interest, and they will constitute a violation of dignity only if the human interests at stake are not greater than the animal's interest.

Under this interpretation, creating "monsters" by genetic engineering—be it true or false, depending on what conception you entertain of a monster—is not a problem if the balance is in favor of human interests.

Ethical Foundation of National Legislation on Animal Experimentation

Among the different ethical views expressed above, legislators have generally incorporated into law a pathocentric view, with its emphasis on reformism. Pathocentrism is realized by the law pointing heavily towards the fact that animals should not be in pain, or experience suffering, stress, or in anxiety when in experiments. Legislation has also incorporated hierarchism in animal experimentation regulations, as "lower" species should replace "higher" ones when the result is comparable.

The Use of Ethical Tools to Assess Mouse Experiments

What kind of tool is used?

How should it be decided whether a mouse experiment is allowed or not, i.e., how to take seriously mice's interests? The ethical tool applied almost universally in animal experimentation is the harm-benefit assessment. The usual image to describe such a tool is the balance, which will compare the torque on an arm weighing the harm to the animal produced by the experiment to the torque on the other arm weighing the benefit generated by the experiment to humans (and/or animals) in terms of health, environment, and knowledge (three overriding human interests).

Instead of the balance, Bateson (1986) proposes a cube with its three dimensions representing quality of research, probability of benefit, and animal suffering, to decide whether a specific animal study should be carried out or not.

Quantification tools have been developed as score sheets (Porter, 1992; Boisvert and Porter, 1995; Stafleu et al., 1999); there is also a Web-based self-assessment score sheet, e.g., *http://tki.samw.ch/*, developed by the Swiss Academy of Sciences.

The process of weighing harms and benefits is often named ethical review process or ERP.

How to undertake the ethical review process?

Although it is easy to understand that the experiment might be acceptable or unacceptable depending onto which side the arms of the balance rotate, or in which part of the cube the project situates, the balance and cube metaphors are misleading. They suppose that the units for measuring harm to the animals are identical to the units measuring benefit for humans and/or animals and quality of research. They suppose that the harm-currency exchange rate is one-to-one with that of the benefit- or quality-of-research currency. This is of course not the case. Moreover, it is difficult to figure out a currency measuring objectively the amount of benefit, the quality of science, or the harm by adding units of benefit, of quality, or of harm. These parameters are partly incommensurable. It is therefore clear that the weighing that one has to do is not a quantitative procedure with mathematical precision. It is rather a question of moral judgment, which will depend on the people doing it. We may compare the process to that of a judge weighing the plea or sentence in the "scales of justice" (Smith and Boyd, 1991).

Who performs the ethical review process?

Countries have established different ways to do an ERP. The process can take place at the level of the institution like the Institutional Animal Care and Use Committee (IACUC) in the United States of America, or at the level of the state, the region, or the country (for Europe, see review by Smith et al., 2007).

It is recommended to do an ERP with a panel of people representing different points of view on animal experimentation. The panel should have a large array of expertise. It should engage in open discussions, and members of the panel should be ready to compromise. Some national regulations call for an ERP done by a single person. This process should always be supervised by a third party. It is also important that the ERP be done locally because it is important to know how animals are used and cared for in the local institutions, as well as the level of training of their researchers and technicians.

Researchers submitting a mouse experimentation project should, in some countries are required to, present their own harm-benefit assessment. Every applicant should go through this assessment to be aware of the ethical issues raised by his/her own work and improve his/her research at the highest ethical standards. The ultimate question that each researcher should ask to him/herself is: "Is it right or wrong to use mice in my project?"

Points that the researcher should consider for a harm-benefit assessment in mouse experiments

Points to review for minimizing harm to the animals: Ideally, the best way to minimize harm to animals is to eliminate it by replacing all in vivo work with nonsentient or nonliving alternatives. As we know, there is a long road to travel before this can be achieved. In the meantime, we should systematically apply the 3R concept: Replace, Reduce, and Refine, that Russell and Birch (1959) (see abridged version in Balls, 2009) have developed and published more than 50 years ago. As we allot efforts to find alternatives to animal experimentation (Replacement), we should at the same time minimize the number of animals we use (Reduction), and improve housing conditions, techniques and procedures that we apply to the animals in order to minimize invasiveness and to improve welfare (Refinement). Much national and international legislation have integrated the 3R concept in order to apply a fair ethical harm—benefit assessment to in vivo studies.

Replacement of animals: First of all, the researcher has to prove that an animal model is an absolute necessity to achieve the aims. A thorough database search has to be presented to convince both the investigator and the ERP panel that this is the best approach to the problem. Whenever possible, replacement-alternatives data should be provided to demonstrate that the animal experiment is the inevitable next step to achieve the intended aims. However, it seems that the research community is not well aware of the available tools to find and review replacement alternatives (Leenaars et al., 2009). An exhaustive literature search in multiple electronic databases is not always simple; advice from librarians may be recommended. Hooijmans et al. (2010a) and Chilov et al. (2007) propose a search filter to collect, respectively, all animal experimentation and alternatives in specific databases such as, for example, the most well-known free database, PubMed (*http://www.ncbi.nlm.nih.gov/pubmed*). Other fee-for-service databases are also available, like Scopus (*http://www.scopus.com*) or Web

of Science (*http://apps.isiknowledge.com*). Through these database searches, researchers may find a better animal model than the one originally in mind, or alternatives avoiding the use of an animal model.

Reduction of number of animals: Russell and Birch (1959) (see abridged version in Balls, 2009) have also insisted on the optimal design of the experiment in order to use the minimum number of animals to achieve statistical significance. An excellent review on design and sample size determination especially devoted to animal experimentation has been written by Festing et al. (2002). However, reduction of number of animals is not a simple matter of statistics. Unnecessary duplication of experiments resulting from poor design, poor training of experimenters, and poor database searches for similar experiments must also be avoided. Therefore, it is important to search genetically modified mice depositories. The KOMP database (*http://www.komp.org/*), the International Knockout Mouse Consortium (*http://www.knockoutmouse.org/*), The Jackson Laboratory (*http://www.jax.org/* and *http://www.informatics.jax.org/*), the EMMA consortium (*http://www.emmanet.org*), and the Japan Mouse/Rat Strain resources database (*http://www.shigen.nig.ac.jp/mouse/jmsr/top.jsp*), among many others, may be consulted (also see *http://www.mmrrc.org/about/resources.html*).

Refinement of housing, procedures, and techniques: Refinement can be defined as "... methods in animal research which alleviate or minimize the pain, distress, or other adverse effects suffered by the animals involved and/or enhance animal well-being." (Smaje et al., 1998). Refinement has to be considered throughout the lifespan, including the death, of the laboratory animals. This is probably the one component of the 3Rs that depends heavily on the training of people for the best practices for housing animals, for procedures, and for application of the least invasive techniques. Highly trained people to run the animal facilities and funding for comfortable housing for the animals and for buying and using bioimaging machinery for longitudinal studies and diagnostic equipment that requires minimal biological sample volume are major contributors to Refinement.

The listing of all possible means to refine animal studies is long. Here we review briefly the major issues in Refinement of mouse experiments:

1. Husbandry and environment of the animals:

• Well equipped animal house, with optimal heating, ventilation and air conditioning (HVAC) equipments.

• Unobstructed flows of clean and dirty material, animal, personal and waste.

• Optimal enriched housing for laboratory animals that spend their entire lives in cages.

• High hygienic standards in animal facilities housing many genetically modified mice coming from laboratories all over the world.

• Least invasive animal identification system.

2. Procedures for the care of animals before, during and after procedures:

• Transport and adaptation to the experimental environment.

• Handling and restraint techniques.

• Animal biopsies.

• Injection and sampling of biological material.

• Anesthesia, analgesia, and surgery.

• Post-operative care.

• Pain identification, evaluation, and alleviation.

• Score sheets to assess humane endpoints and to take actions. Consultation of genetically modified mouse databases which incorporate issues on deleterious phenotypes can greatly help in creating such score sheets (see also *http://www.eumodic.org/*).

• State-of-the-art euthanasia.

The specific costs of creating and using genetically modified mice

Worldwide statistics on the number of animals used in experimentation show the preeminence of the mouse model; this species is very well adapted to genetic manipulation, reproduces rapidly, and is easy to house. The genetic model is often far more predictive when testing hypotheses regarding fundamental biological processes or when mimicking human diseases than the classical models used in the past. This signifies that a refinement has been achieved. However, we have to remember that to get this higher-quality (genetically engineered) animal model, many animals have been generated and euthanized during the process. Therefore it is crucial to review databases on genetically modified mice to search for the model of interest. This saves time, money, and animals. However, the GM model approach may in some circumstances create a conflict between the Refinement and Reduction

component of the 3Rs that the ERP has to solve. It is therefore very important to review the specific costs linked to genetic engineering of mice and to take appropriate actions.

Costs are of three kinds:

1. the *procedures* used to create the GM line;

2. the *husbandry* to establish and to maintain the GM lines which generate often a large number of animals that have to be discarded;

3. the possible negative impact of the *genetic manipulation* on the welfare of the individual animals.

The review on "refinement and reduction applied to the production of GM mice" published by Robinson et al. (2003) gives excellent advice, from creation to archiving of GM mice, and for implementation of best practices in the lab and the animal facilities (see also Australian Animal Welfare Committee, 2006).

Before starting any creation of a GM mouse line, researchers have to make sure that the GM mouse of interest is not already available (see under section "Reduction of number of animals," listing several mice databases). If not, the genetic engineering process may start with an appropriate design of a transgene.

At this stage, *procedures* to create the GM line have to be reviewed and the following main issues have to be considered:

● Selection of mouse strains appropriate as embryo source; high plug rates; implantation; and survival of embryos and pups.

● Protocols for superovulation.

● Surgery, anesthesia, analgesia, and postoperative followup for best practices of vasectomy and transfer of embryos to foster mothers.

● Size/weight/age optimization of female embryo donors and selection of the least aggressive but still sexually aroused stud males.

When founders are available, expansion and maintenance of the GM lines raise *husbandry* issues. The following have to be addressed:

● Best practices in colony management.

● Implementation of high hygiene standards to prevent health problems.

● Pair housing of pregnant mice receiving eggs of the same microinjection experiment instead of single housing.

● Enrichment of cages housing single vasectomized and stud mice.

● Choice of the least invasive methods to take biopsies for genotyping and identification of animals. When both genotyping and identification of the same individual are required,

consider the dual-purpose approach (ear punch or phalange amputation).

● Selection of training of personnel for the most appropriate euthanasia procedures for culling GM mice colonies.

● Sending cryopreserved embryos or sperm instead of live animals for inter-laboratory exchange, for purposes of welfare and hygiene.

● Archiving GM lines by cryopreservation of embryos, sperm or ovarian tissue.

Finally, to minimize welfare problems linked to the *genetic modifications*, the following have to be considered:

● Use of inducible promoters and conditional transgenes to avoid life-long genetically linked welfare problems.

● Breeding of homozygotes if no welfare problem is detected.

● Phenotyping of new lines from birth onward for early detection of welfare problems.

● If welfare problems arise, set clear humane endpoints to avoid excessive harm to the animals.

Maximizing the benefits to humans or animals

To justify the harm inflicted upon the laboratory animals, researchers should answer two questions: (1) What is the contribution of my research to the improvement of human (and/or animal) health; and (2) in which timeframe will I achieve the short-, medium-, and long-term aims of the project? Additionally, they should adhere to the duty of following the highest scientific quality standards. Below, "quality of research" is defined. Relevance of research to society and opportunities for reaching specific aims depend heavily on directions taken by society, politics of science, granting agencies, and objectives of large institutions and laboratories. This debate involves every researcher.

Quality of science

Peer review is the quality-control measure that scientists use to assess quality of their research. It correlates funding to publication of scientific papers in high-impact journals. A published paper of in vivo studies is just the tip of an iceberg of information including the housing and care of animals in facilities, experimental procedures on living beings, study design, allocation of animals to different experimental groups, blinded analysis of biological samples and experimental outcomes, etc. A survey made by Kilkenny et al. (2009) on research using animals shows that reporting

is often incomplete and not transparent and that design and statistical methods are poor. Lack of accuracy and transparency, and incomplete reporting in animal experimentation, make review and meta-analysis of research fields almost impossible (Hooijmans et al., 2010b; Kilkenny et al., 2010). It means that, often, clinical-trial design cannot be backed up by animal-model results. Such situations further muddle the already controversial field of animal experimentation; moreover, it hampers the benefit side of the harm-benefit assessment of animal experiments. Kilkenny et al. (2009) and Hooijmans et al. (2010b) show also that researchers have to work directly with animal facility teams to grasp the entire complexity of an animal experiment. Therefore, there is an ethical need to perform high-quality work in the lab as well as in the animal house.

Progress to achieving high quality science in the field of in vivo studies can be made on the lab side by applying the ARRIVE initiative (for Animal Research: Reporting In Vivo Experiments; Kilkenny et al., 2010) and/or use the checklist for animal experimentation reporting issued by Hooijmans et al. (2010b) from the 3R Center of Radboud University, Nijmegen, The Netherlands.

For animal housing, implementation of a quality control procedure is helpful for achieving high-quality housing and care of laboratory animals (Dirnagl, 2010).

CONCLUSIONS

Animal experimentation is an important aspect of life science and medical research. It is even mandatory for drug development. Animals, however, are not things we can use as we please; they have a moral standing because they possess interests that can be thwarted. In short, human beings can harm them. From an ethical point of view, harming animals is not impermissible, but ought to be justified. Current legislation of animal protection is pathocentric in essence: human and animal interests have to be weighed to decide whether an animal experiment is morally justified. Experiments with mice must therefore go through a harm-benefit assessment, with the 3Rs being a prerequisite to such assessment. But all animals are not on the same footing; our societies tend to adopt a hierarchical concept of animals, where certain animals count for more than others. For instance, mice have the capacity to suffer and to flourish, but they do not have a mental life as rich as apes or other nonhuman primates. Therefore, their moral standing

is lower, and experiments with this species are easier to justify morally than the same studies on nonhuman primates such as apes.

In a reformist perspective of animal experimentation, the 3Rs approach develops its full strength by obligating scientists to think through their experiments thoroughly. In conclusion, ethical considerations with respect to mouse experiments do not only require consideration and improvement of animal welfare, but also improvement of the quality of science.

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APPENDIX: GLOSSARY

Anthropocentrism: A moral theory is anthropocentrist if it puts human being at the top of the moral realm. Usually it gives moral status only to human beings (but see hierarchism).

Autonomy: A being is autonomous if he can choose between various possibilities and act accordingly, i.e., freely. Traditionally, autonomy requires the capacity to ponder about one's own desires (see Reason), but certain authors attribute autonomy to animals, as they can act accordingly to their desires or preferences.

Biocentrism: A moral theory is biocentrist if it believes that the morally relevant intrinsic property is life. For biocentrism, all living beings possess moral status.

Dignity: Dignity is synonymous with intrinsic value, and denotes a high intrinsic value. It has therefore traditionally been kept for human beings.

Harm-benefit assessment: This is a weighing between the likely adverse effects on the animals and the benefits to human beings or other species likely to accrue as the result of the research.

Hierarchism: A moral theory is hierarchist if it believes that there exist several moral statuses ranked on a scale of value. Usually it puts human beings at the top of the scale, then animals followed by plants, etc.

Indirect duty: A person has an indirect duty towards a being (e.g., an animal) if this being is only an opportunity for the person to fulfill a direct duty towards another being (e.g., a human person).

Intrinsic property: A being possesses two kinds of properties, intrinsic and extrinsic. A property is intrinsic if it belongs to the being independently of his environment; in any other case, the property is extrinsic. "To have four legs" is an intrinsic property for a rat, whereas "To be bigger than a mouse" is an extrinsic one.

Intrinsic value: A being can have two kinds of value, intrinsic and extrinsic. His value is intrinsic if it depends on his morally relevant intrinsic properties; in any other case, his value is extrinsic. "To be sentient" gives an intrinsic value to a rat, whereas "to be loved" gives an extrinsic one.

Moral agent: A being is a moral agent if he has moral duties towards other beings; he is a moral patient if he has only moral rights and no moral duties. Adult human beings are moral agents, children and, for certain authors, animals, are moral patients.

Moral status: This denotes the place that a being occupies in the moral realm. The place of a human being is different from the place of a rat. Their moral status is therefore not the same. To determine a being's moral status, we must take his morally relevant intrinsic properties and his intrinsic value into account.

Pathocentrism: A moral theory is pathocentrist if it believes that the morally relevant intrinsic property is sentience, i.e., the capacity for suffering and for happiness. For pathocentrism, only sentient beings, i.e., human beings and animals, possess moral status.

3Rs: Described first by Russell and Birch (1959), three principles that guide the use of animals in experimentation: (1) replacement (use of alternatives to animals whenever it is possible to reach the same scientific goals); reduction (use of minimal number of animals to obtain scientifically significant results); and refinement (use of any method and procedure to decrease or eliminate pain, suffering, distress, fear, or anxiety, and to increase the welfare of animals that cannot be replaced by other methods).

Reason: This is a multifarious capacity that comprises in particular autonomy, self-consciousness, conscience, and self-mastery. Traditionally, this capacity is kept for human beings.

Sentience: This is the capacity for suffering and for happiness. For pathocentrism, this capacity determines moral status.