The Question of Animal Experimentation

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articolo

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"I am life that wants to live, in the midst of life that wants to live"[1]. Albert Schweitzer's sentence outlines the ideal approach to all living beings including animals. But what to do when confronted with the so-called "life-boat situation"; save human or animal life? Such an ethical dilemma is at the heart of animal experimentation.

Using animal studies in the development of life-saving drugs such as antibiotics and vaccines, in testing the safety of food, working material, or material released into the environment, has substantially contributed to longer human life expectancy. Until the 1970s, ethics in medical research focused on protection of the human subject and the good of the patient, heralding the Hippocratic rule of "do no harm" to man as the most important principle. Guidelines on the ethics of clinical research, such as the Nuremberg Code, the Declaration of Helsinki, and the Belmont Report stress the protection of human health and dignity.

Increasing concern for animal welfare, however, questions the rationale and usefulness of animal studies. Society and legal initiatives require a balance between the protection of animals and that of humans. This ethical dilemma has to be faced by the researcher himself, by research institutes, by the pharmaceutical and chemical industry, and finally, by the consumers of medications and chemical products.

This article provides insight into the complex environment involved in ethical decision making and an overview of the areas of research in which animal experimentation is performed; it also introduces basic principles and regulations for animal welfare and ethical review.

1. The complexity of ethical decision making

In 2014, the European Union Committee of Experts on Rare Diseases (EUCERD) estimated that as many as 5,000 to 8,000 distinct rare diseases exist. The total number of people affected in the EU is estimated at 27 to 36 million[2]. These patients are given hope by positive results from animal experimentation. In addition, each disease outbreak that poses a threat to global health, such as the recent spread of Ebola, leads to public demand for quick and safe drug development including animal research.

Despite the continuous demand for animal use in research and drug development, parts of society are increasingly rejecting animal experimentation due to a lack of information, doubt of success, and moral outrage especially by animal rights activists accusing researchers and industry of performing animal studies for profit only. These groups misjudge the situation. In most cases, researchers and industry are an interface between science, technology, public and ethical demands to protect patients and animals; they are also required by international and national law and regulations to carry out animal experimentation.

The following paragraph describes the crucial factors for ethical judgement in non-

clinical drug development and assessment of chemicals for ecotoxicology and biodegradation (see Figure 1). The major agents for drug development and basic research are academia, Contract Research Organisations (CROs), and industry. The means to gain valid results and information include *in vivo* and *in vitro* research. Legislations and regulatory guidelines heavily restrict and control the approval process necessary to market a drug or a chemical compound.

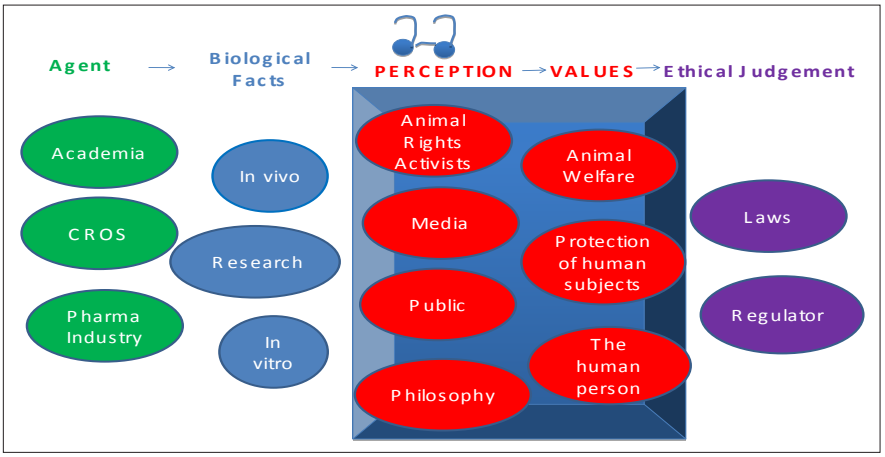

Figure 1: Relevant factors in ethical judgement in animal research.

How one perceives research and *which values to include for consideration* are a critical step in ethical judgment in research. According to Thomas Aquinas, the truth can only be found when reason and fact are in agreement, i.e. when one sees things as they really are: *Veritas est adaequatio rei et intellectus*[3]. J. Pieper calls this *"seinstreues Gedächtnis"*[4]. But the truth of reality is often masked by hidden agendas. Legislation's and industry's perception on research is often shaped by background decisions. These preliminaries are like coloured glasses which skew our point of view. The ethical perspective is influenced by the public, by animal rights activists, media, and by philosophical currents. Table 1 outlines some ethical/philosophical ideas and their consequences for animal experimentation. These preliminary decisions determine to which extent certain values are included into ethical judgement on animal experimentation. Is it simply animal welfare? What role does the protection of human subjects play? Do animals have rights and dignity similar to man? Observing animals, we are *inclined* to care for, respect, and even love them. We disapprove of any cruelty in our feelings. In a further step, our *reason/intelligence* submits our inclination to an analysis of values, which may be of biological, psychological, or cultural bent. Instead of being open to the whole of reality, however, animal welfare and status is influenced by philosophical reasoning, which derives from utilitarian approaches and materialistic evolutionism, and a misinterpreted anthropocentrism resulting in irresponsible actions against animals[5]. Being open to the whole of reality means to understand the scientific basis and need for animal studies in diverse areas.

Ethical concept	Basic Research	Drug Development	Ecotoxicology	Assessment of chemicals
Animal Rights (Regan 1989)	No	No	No	No
Pathocentrism/ Utilitarism (Singer 1986)	No	No	Possible in emergency	Possible in emergency
Anthropocentism (Kant 1788)	Yes	Yes	Yes	Yes
Personalism (Sgreccia, 1999)	Yes, if benefit for man	Yes, if benefit for man	Yes, if benefit for man	Yes, if benefit for man

Table 1. Ethical/philosophical currents and consequences for animal experimentation
Assessment of the categories by Kant reflects his assumed opinion.
No= rejection, yes=acceptance of animal experimentation

2. Different areas involving animal experimentation

Animal studies are mandatory for drug development, approval of chemical products, and ecotoxicological assessment.

REACH, the European Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals ensures a high level of protection of humans and the environment from health risks posed by chemicals[6]. Often, only animal studies can verify the safety of chemicals. Pre-clinical *in vivo* studies must meet ecological and nature preservation criteria and are required for registration and market approval of chemicals, agrochemicals, and biocides.

An example for the necessity of animal studies is the ongoing discussion on the toxicity of endocrine disrupting chemicals. These compounds include a wide spectrum of substances which act on the endocrine system, disrupting its normal function and thus causing adverse health effects in human and animal organisms. Synthetic hormones, such as drugs, plastic compounds, compounds used in textiles and pesticides are just a few examples of the diverse sources of ecotoxicants.

An experiment showed that only 5 parts per trillion of ethinylestradiol, a contraceptive drug, when poured into a Canadian lake, killed all the fish therein[7]. Animal studies in rodents with Bisphenol A during early development revealed the carcinogenic effect of this chemical[8,9]. Trout, carp, zebra fish, earthworms, honey bees, and Japanese quail are often used for ecotoxicological risk assessment[10].

Today, drug development involves more complex issues than for traditional pharmaceuticals on the market. It takes 12 years on average to develop a new product, and only 1 in 10,000 compounds reach market approval[11,12]. Recent advances in science, but also regulatory authorities, require more and more data and consequently, more studies in animals. Laboratory animals are not only used for efficacy and safety evaluation of a new medicinal product, but also for quality batch control testing as part of the manufacturing process. Regulatory authorities and international guidelines such as those of the ICH (International Conference on Harmonisation) and the ISO (International Organization for Standardization) standards 10993 require thorough safety and efficacy assessment of a potential drug candidate or medical device for first-in-human dose studies and market authorization of a new drug[13,14]. Risk-benefit assessment of drugs for humans still relies heavily on non-clinical safety and efficacy studies performed in animals. For safety testing, mice, rats, rabbits, dogs, mini pigs, and non-human primates (NHPs) such as cynomolgus and rhesus monkeys are used, depending on the classification of the drug candidate, cross-reactivity with the different species, and regulatory requirements. Efficacy is preferably tested in mouse models, as mice can be easily genetically modified with human genes to reflect human disease.

Basic research is required for progress in drug research, ecotoxicology, and toxicological assessment of chemicals. Gaining knowledge of biological pathways, function of receptors and enzymes, and design of disease models to mimic human diseases relies heavily on animal studies. Despite the scientific need for animal studies, all such experimentation must be based on sound justification that balances the benefits for humans/animals derived from animal studies against the harm inflicted on them. Only a major benefit to humans can justify a study in animals. The demand for animal welfare is reflected by a growing number of regulations which restrict suffering and number of animals to a minimum.

3. Regulations and principles of animal welfare for animal experimentation

3.1. History of regulations

Since the 1970s, numerous animal welfare laws and guidelines have been implemented into US law and EU legislation and that of their member states.

In the US, the Animal Welfare Act was brought into effect in 1966. It is the only Federal law in the United States that regulates treatment of animals in research, exhibition, transport, and by dealers. Other laws, policies, and guidelines might include additional specifications for animal care and use, but all refer to the Animal Welfare Act as the minimum acceptable standard[15]. In addition, the Guide for the Care and Use of Laboratory Animals has been a respected resource for decades[16]. An accreditation committee, the "Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC)", was formed in 1963[17]. Most American pharmaceutical companies, but also more and more companies all over the world, volunteer for accreditation and regular inspection concerning animal welfare by AAALAC committees.

In Europe, in 1986, the Council of the European Communities adopted Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes[18]. In brief, this directive aimed to maximise animal welfare and reduce to a minimum the number of animals used in studies for scientific purposes. Furthermore, it required a guarantee of animal welfare in experiments, as far as general care and accommodation is concerned[19]. In 2007, new Guidelines for Accommodation of Animals (European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes- ETS 123 2007) became effective. These guidelines define the species-specific provisions for laboratory animals including NHPs and set European and international standards, especially for social housing and future refinement[20].

3.2. Current EU Directive

Directive 2010/63/EU, which replaced Directive 86/609/EEC in 2013, aims to harmonise animal welfare for animals used for scientific purposes in all EU member states. Whereas countries such as Great Britain, Germany and Austria already had strict animal welfare regulations in place, southern and eastern countries have been well behind these requirements.

The new Directive stresses that more efforts are needed to devise alternative methods to animal testing. The legislation is presented as "an important step towards achieving the final goal of full replacement of procedures on live animals for scientific and educational purposes as soon as it is scientifically possible to do so"[21]. All Member States must ensure that whenever an alternative method is recognised by Community law, it is used instead of animal testing. Increasing funding for projects aiming to replace, reduce, and refine the use of animals for scientific experiments is provided by the Community Framework Programmes for Research and Technological Development[22]. If animal studies cannot be avoided, the choice of species for scientific experiments is crucial, especially when considering the most evolved animal species, NHPs.

Therefore, a second important topic deals with using fewer NHPs in pre-clinical research. A proposed ban on scientific testing on great apes such as chimpanzees and gorillas was generally endorsed by committee members. However, these measures would also restrict the use of macaques for example, and thus hamper European scientific research on neurodegenerative illnesses including Alzheimer's and chronic autoimmune diseases, especially for the development of biologics[23]. As a consequence, the Directive allows the use of NHPs only when there is scientific evidence that testing in these species is necessary to achieve the objective of the programme and to benefit human beings. The use of NHPs is also a very sensitive topic due to these animals having a high capacity to experience pain, suffering, and distress. The legislation introduces categories of pain inflicted during a test ("non-recovery", "mild", "moderate" or "severe") for all species. To avoid repeated suffering, the Commission has proposed to allow the same animals to be reused only when the previous experiment entailed pain classed as "up to moderate". Furthermore, the Directive covers the protection of mammalian foetuses especially

in the third trimester. An upper limit of pain is required, and death as an endpoint of a study should be avoided[24].

The EU Directive also requires assessment of the harm inflicted on animals during a study relative to the benefit for humans as a result of such experimentation. The directive cedes the method of analysis to the EU Member States, which has initiated heated discussions between the different parties of interest. The parameters for assessing the benefit not yet defined remain subjective. Which benefits are most relevant: Social, scientific, economic, or educational? Even the European Commission admits that no single system of analysis will do justice to all projects[25].

In the Directive 2010/63/EU, care of animals is based on the principles of "replacement, reduction and refinement" which were first published by W.M.S. Russell and R.L. Burch in 1959[26]. These principles are also called the "3Rs" and comprise *replacement* of animal studies by alternative methods, *reduction* of the number of animals used in an experiment, and *refinement* of techniques used in order to decrease the incidence or amount of animal pain and distress.

3.3. The cornerstone of animal welfare - the 3Rs

The value of life is fundamental in animal experimentation. The concept of animal rights, mainly influenced by T. Regan, virtually bars all use of animals in research because they are "subjects-of–a-life"[27]. In Regan's deontological perspective, animals, like humans, have a right to live which cannot be weighed against human interests[28]. This thinking is reflected in the new EU Directive's ultimate goal "of full replacement of procedures on live animals"[29]. In vitro cell-based technologies, computational modelling, and high throughput techniques are used to replace animal testing. If a study is designed to test toxicity or safety pharmacology of a drug candidate, the proposed alternative method must be validated by international organisations, such as EURL ECVAM (The European Union Reference Laboratory for alternatives to animal testing), in order to be approved by regulatory agencies[30]. To overcome the discrepancies between in vitro systems and the human body, the EU has supported projects with human embryonic stem cells[31]. In the meantime, many assays use induced pluripotent stem cells (iPS); however, e.g. cardiotoxicity assays still involve human embryonic stem cells[32].

To replicate the organ complexity of the human body, tissues-on-a-chip are currently being developed with the aim of producing a human-on-a-chip. Advances in bioengineering and material sciences have led to the development of microsphysiological systems that mimic the functional units of an organ like lung, heart, or liver[33,34]. One major objective of animal welfare is to avoid unnecessary animal experimentation, for example, duplication of animal studies[35]. Careful design of studies based on statistical analysis must ensure that the number of animals per treatment group provides reasonable results. Any attempt to reduce the number of animals in research, however, should not divert from the fact that these are sensitive beings. Prevention of suffering is at the heart of all animal welfare. Animal welfare guidelines prescribe the use of species and procedure specific analgesics and anaesthetics.

Despite granting animals the same moral status as humans due to their sensitivity to pain, P. Singer distinguishes between more evolved animals (monkeys and dogs) and lower animal species (rats, mice, and fish): The closer the evolutionary proximity to humans, the more morally relevant the animal[36]. This discrimination is also found in animal welfare legislations. Whereas the use of chimpanzees is forbidden by the EU Directive, and careful consideration is required for inclusion of other NHPs in research, experimentation with rats, mice and fish can more easily be ethically justified[37].

Is there really a difference between rats or dogs though in terms of suffering and moral status? Has not each animal an intrinsic value? Recent research shows pain-like states even in some molluscs[38]. Therefore, suffering must be limited by defining humane

endpoints for all animal species. Common humane endpoints are a decrease in body weight by a certain percentage and a defined maximum tumour volume in xenograft cancer models in mice. Such outcome measures require extensive training of all personnel involved in the performance of a study, especially of the animal caretaker/technician, who develops the closest bond with them[39]. Refinement rules include improved housing, such as a defined cage size, freedom of movement, social contact, meaningful activity, nutrition, and water, with restrictions only for a minimum of time and degree. Laboratories must provide social/group housing for animals. European facilities offer cages for group housing of monkeys, which can be as high as three stories, and allow visual, acoustic, and/or olfactory contact between animals in different cages[40].

Day-to-day animal care involves the relationship between caretaker/technician, veterinarian, and animal. R.L. Walker discusses the concept of flourishing for animals, which includes a more comprehensive understanding of their well-being[41]. For Walker, an animal "flourishes when it lives a life that is good for it, both as a particular kind and as a specific individual, where notions of "good for" are taken in part from a view of what is natural for it, and are assessed over its lifetime" [42]. This type of care can only be met by practicing virtues such as practical wisdom, patience, respect, care, friendship, compassion, justice, and reliability, depending on context[43].

An example for exercising patience and respect regarding the intrinsic value of each animal is by carefully assessing primates' social ranking. Aggressive and submissive behaviour should be monitored. Animals that do not exhibit submissive behaviour are considered alpha animals[44]. With time and daily interaction, the caretaker knows which of "his" animals are dominant, intermediate, or low ranking.

While 3R initiatives are crucial, they become meaningless without a balance between the ethical justification for a project as a whole and a detailed scrutiny of procedures[45].

4. Ethical review process

Over the last 10 years, most companies have begun to incorporate animal ethics and welfare into corporate (bio)ethics policies, position statements, and annual sustainability reports. Independent animal welfare officers ensure that animal welfare is observed during the housing and experimental phase. Animal ethics committees, also called the Institutional Animal Care and Use Committee (IACUC), review animal study protocols. These committees consist of veterinarians, animal users, scientists of relevant research areas, independent lay persons, and (bio)ethicists when available. All animal studies must be approved by governmental agencies. Animal facilities and programmes are regularly inspected by government agencies. Studies that are outsourced to CROs, as done by an increasing number of companies, must be also ethically justified, reviewed by an internal ethics committee, and regularly audited for animal welfare. Most of these processes are incorporated into the companies' quality system as Standard Operation Procedures (SOPs).

5. Ethical considerations for animal research in drug development

The procedure-driven ethical approach described above should be supported by constant in-depth ethical and philosophical reflection. The basic dilemma of drug development is who should be saved: Man or animal?

The document on Prospects for Xenotransplantation of the Pontifical Academy for Life states: "there should be a reaffirmation of the right and the duty of man … to act within the created order … in order to achieve the final goal of all creation …. The sacrifice of animals can be justified only if required to achieve an important benefit for man. … However (in every) case there is the ethical requirement that in using animals, man must observe certain conditions: unnecessary animal suffering must be prevented; criteria of

real necessity and reasonableness must be respected; genetic modifications that could significantly alter the biodiversity and the balance of species in the animal world must be avoided"[46].

The same document also affirms that man transcends all living beings: "it is man who has always directed the realities of the world, controlling the other living and non-living beings according to determined purposes"[47]. This reflection is based on Thomistic personalism according to Boethius` definition: "Persona est naturae rationalis individua substantia" [48] implying that man has an inalienable and intrinsic dignity which is rooted in his rational nature. Between man and the rest of creation exists a gulf precisely because of his rational and spiritual nature, which finds its expression in his freedom, creativity, self-consciousness, and interiority[49]. Only man can be the subject of ethical responsibility. Only humans can be object and subject at the same time. Whereas the objectivity of an individual is connected to the assumption of reducibility of the human to the world, subjectivity means "that the human being's proper essence cannot be reduced to and explained by the proximate genus and specific difference. Subjectivity is, then, a kind of synonym for the irreducible in the human being"[50].

J. Maritain, who contributed to the drafting the United Nations Universal Declaration of Human Rights in 1948, stated that personality "signifies interiority to self"[51]. This means that "the person differs even from the most advanced animals by "a specific inner self, an inner life" which revolves around truth and goodness"[52]. Jane Goodall, the world's foremost expert on chimpanzees said that, in contrast to man, chimpanzees do not ask for the sense and truth of life[53].

Indeed, man is the only animal who contemplates possible life after death[54]. The animal is trapped by his instincts in the present moment. It has all the time in the world to perceive and observe. R. Hagencord considers the animal's life in the present moment a challenge for the modern human being. Man has lost the true perception of reality and is very often guided only by intellectual reasoning which prevents him from recognizing the challenges of the moment[55]. Indeed, the openness of reality should be the basis for animal welfare and ethical requirements in the pharmaceutical industry.

5.1. An opportunity for more scientific in-depth reflection[56]

Today's drug development is heavily restricted by shareholder value and, therefore, by narrow timelines and the requirement to obtain market approval as quickly as possible. Studies involving animals are often initiated without sound scientific justification. In addition, the pressure on scientists to publish as many papers as possible results in studies with inconclusive outcome. The importance of having sufficiently high numbers of animals in the study for statistical analysis and the risk/benefit analysis for human subjects must be taken into account. Simple reduction of animal numbers under the pressure of animal rights activists without regard to the project's final objective will either put human safety at risk or require a repetition of the study, thus significantly enhancing the number of animals used. Therefore, careful reflection on the selection of relevant animal disease models and on possible combinations of safety/efficacy/pharmacokinetics and toxicology studies is necessary. Ethical evaluation requires not only assessment of the possible harm of animals, but also of the importance and benefit of the study for the whole project.

5.2. An opportunity for in-depth ethical reflection on changing the life of man and animal

The European Directive on animal welfare requires from the pharmaceutical industry, CROs, and research institutes a harm/benefit assessment for studies and projects including animals[57]. The Federation of European Laboratory Animal Science Associations (FELASA) provides valuable principles for a thorough ethical evaluation and review of animal experiments[58]. Three key points

are especially interesting to note: First, ethical evaluation of scientific projects must take into account the overall objectives of the project. For this purpose, a wide enough range of expertise is of vital importance to understand the *whole of reality*. Secondly, "factors for consideration" are regarded as valuable. However, ethical evaluation can never be reduced to checking boxes. Ethical evaluation is *not a mechanical method*. Ethical review must be a *dialogue* and can evolve with *experience*[59]. Indeed, drug development is more than just production of a drug. The requirements for animal welfare remind us that each decisive step in drug development may change the life of man. There is a significant difference between acting and producing, between *praxis* and *poesis*. Whereas a simple technical activity "remains outside" of the actor, the act as an *operatio immanens* stays within him and changes his life and the life of human patients[60]. Reflections on the strategy of drug development influence not only the quality of the drug product, but also the selection of therapeutic areas. This new view of animal welfare will necessitate revision of drug development strategy as a whole.

5.3. An opportunity for further innovation tied to the happiness of man and animal

The market is what drives drug development. Current utilitarian focus on health enhancement and life-style drugs often creates a need by "condition branding" that threatens to confuse the well-being of the human with that of the market[61]. For the development of such medications, experimentation on animals is not ethically justified. In addition, using animals to develop "me-too-drugs" involving only a minor improvement in benefits for patients is ethically questionable with respect to both animal and patient: Both animal and human life will be harmed by a drug development strategy focused only on life-style and "me-too". The objective of animal ethics is to remind us that human and animal life is connected. According to the Old Testament, both man and animal have a soul (*nefäsch)*, i.e. a longing for happiness. The Latin word *anima* refers to "animal". This reminds man that life is a gift and ties his happiness to that of all other creatures[62]. Man experiences real happiness when he realizes his creative and innovative powers[63]. Therefore, more courage is required in developing drugs that are urgently needed in the Third World, that are life-saving or indicated for rare diseases, or that improve the life of chronically diseased patients.

Conclusion

What animal welfare needs today is courage. Courage to find new ways of ensuring animal well-being within drug development and ecoteoxicological assessment rather than reacting to pressure by animal rights activists. Man's freedom from instinctive re-activity is also his responsibility. Without an awareness of his intrinsic dignity, however, no ethics can exist which lead to the happiness of man and animal.

NOTE

* The author works as manager in the pharmaceutical industry. The article reflects her personal opinion.

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