Chapter 10
How Technology Can Replace Animals in Lab Practices

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In the permanent struggle between instinct and culture, science and education can help us combat ignorance. Through education, we receive in a way the cumulative universal culture from the past to the present. We also have developed social patterns that enable us to find finding out our personal space within our respective communities. Curiosity and the extension of the knowledge is a human characteristic. We always want to know more, we solve problems in order to survive, and we imagine possible alternatives. Science helps us to progress and has the power to questioning itself every day, it takes us forward in the reasoned explanations of conditions and it offers possible solutions to the multiple challenges we encounter as a species. One of these challenges is that of developing a keen awareness that we are not the only important species on Earth. Peter Singer (2005) promotes in his works the concept of *speciesism*, noting critically human intolerance or discrimination among species. The net result of this concept is the tendency for humans to thing that all non-human animals are intrinsically inferior to humans, just because they are not humans. Such perspectives are easily refutable from a biological point of view. Intelligence is measured on a continuous scale. At the lowest level in animals we find the slime mold, a unicellular organism that is halfway between *fungus* and animal. Though it lacks a nervous system, a slime mold still finds the shortest path through a maze and can interconnect two food sources, which evidences, as Nakagaki says (2000) that it is able to process information. From this minimal

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evolutionary structure it is unquestionable to claim that all animals, humans too (even though sometimes it may not seem true), have intelligence at varying grades.

As a species, we do not treat well at all other species. We do domesticate and breed hundreds of animal species for food or transportation, and use them for entertainment (circus animals, cock fighting, bullfights), we hunt them (deer; fox; rabbits; elephants), we use them for clothing and accessories, exhibit them in zoos, and increasingly, by means direct and indirect, we have driven many species to extinction. We also care for them, protect them, and even adopt them as members of their own families. And these people are precisely those who most closely are questioning that science even today continues to use animals in research and clinical trials.

The scientific arguments for using animals in research are clear. Clinical trials with animals allow us to develop hypotheses about living organisms to find possible solutions to problems related to our health and quality of life. From one point of view or another, the debate on animal research remains polarized between those in favor and those against all animal research (Perry 2007). Animal testing is based on procedures performed with live animals for research purposes. The animals most commonly used for such purposes are: mice, rats, birds (pigeons), rabbits, guinea pigs, hamsters, fish (zebra fish is the common model), farm animals such as pigs, sheep, and chickens), dogs, primates (including orangutans, chimpanzees and bonobos), cats, and frogs. Most of these animals are purpose-bred, that is, specifically bred to be used for experimental purposes in areas as diverse as teaching, industry or biomedicine. In teaching, animal experiments in basic biology, animal behavior or medicine can help students gain knowledge of the physiological, morphological, behavioral and anatomical characteristics of living beings. In industry, animals are used to check the safety of materials in various household products (such as cosmetics, cleaning agents and food additives) and lawn-care and agrochemicals. Animals also are used in military tests, and in the biomedical domain, tests with animals are used to evaluate the effectiveness of new drugs to treat diseases, and to conduct research in diverse areas of medicine, dentistry and veterinary. Liebsch et al. (2011) estimate that annually, more than 115 million animals worldwide are used in lab experiments (Humane Society International 2015). The consequences for the animals used in such experiments are often very severe. The pain inflicted on an animal should follow the principle of analogy, that is, that the same pain caused to the animal could be suffered by a human, and pain will be infringed only if it cannot be excluded from the test (Hedenqvist and Hellebrekers 2003). But we must not be naïve, all procedures, including those classified as mild, have the potential to cause physical injury, psychological distress and suffering. Most of these animals are euthanized and sacrificed at the end of an experiment; a few may be re-used in subsequent experiments.

10.1 Animal Testing

In Europe, since 1985, there are laws to protect vertebrate animals used in scientific experiments. An example is the Directive 2010/63/EU that establishes basic rules for protecting animals that are bred for scientific use, and other purposes, including teaching. The following rules are from this Directive: minimize the number of animals used in procedures, and wherever possible use alternative methods; do not cause the animals unnecessary pain, suffering, distress or lasting harm; avoid any unnecessary duplication of procedures; and ensure that the animals have the proper care. In 2010, the European Commission published data on the number of animals used in experiments by the Member States of the European Union. These data showed that in 2008, mice, rats and rabbits accounted for more than 80% of the total number of animals used in laboratories. These three groups of mammals were followed by various poikilothermic animals (reptiles, amphibians and fish), which accounted for 9.6% of the animals used. Birds represented 6.3% of all animals used. At a finer scale, the vertebrate animals used in these procedures were: Mouse (*Mus musculus*), Rat (*Rattus norvegicus*), Guinea pig (*Cavia porcellus*), Golden hamster (*Mesocricetus auratus*), *Chinese dwarf hamsters* (*Cricetulus griseus*), *Mongolian gerbil* (*Meriones unguiculatus*), Rabbit (*Oryctolagus cuniculus*), Dog (*Canis familiaris*), Cat (*Felis catus*), all species of nonhuman primates, F*rogs* (*Xenopus laevis*, *X. tropicalis*, *Rana temporaria*, *R. pipiens*) and zebrafish (*Danio rerio*).

The European Commission report indicates that both fundamental biology and research and development in human and veterinary medicine are, by far, the fields that use the largest number of laboratory animals (Fig. 10.1). The number of animals used for research and development in medicine, dentistry and veterinary declined from 3.7 million in 2005 to 2.7 million in 2008. Over the same time interval, the percentage of animals used for fundamental biological research increased, from about 4.0 million in 2005 to nearly 4.6 million in 2010. The number of animals used for toxicological evaluations and other safety assessments in the European Union remained unchanged, at about 1.0 million per year.

From the data provided by the report we can see a substantial increase in the use of mice and rabbits for the production and quality control of products and medical instruments and dentistry, as well as some increase in the use of mice, pigs and birds for basic biological research, anatomy, developmental biology, physiology, genetics and cancer research, immunology and microbiology. It seems that the increase registered in the use of mice is attributed to the new research possibilities offered by the transgenic species, used for studies about health.

10.2 Ethics in Animal-Study Research

Anyone who cares for or uses animals in research must assume responsibility for their general welfare (National Research Council 2011). This objective is feasible, but we should not forget that the general welfare of animals can be contradicted by the fact that in the end, they are killed, although “as humanely as possible”. There it is important to note that there are rules governing research objectives when they involve animals in testing (Resnik 2012). The International Committee for Laboratory Animal Science (ICLAS) brings together some 100 countries around the

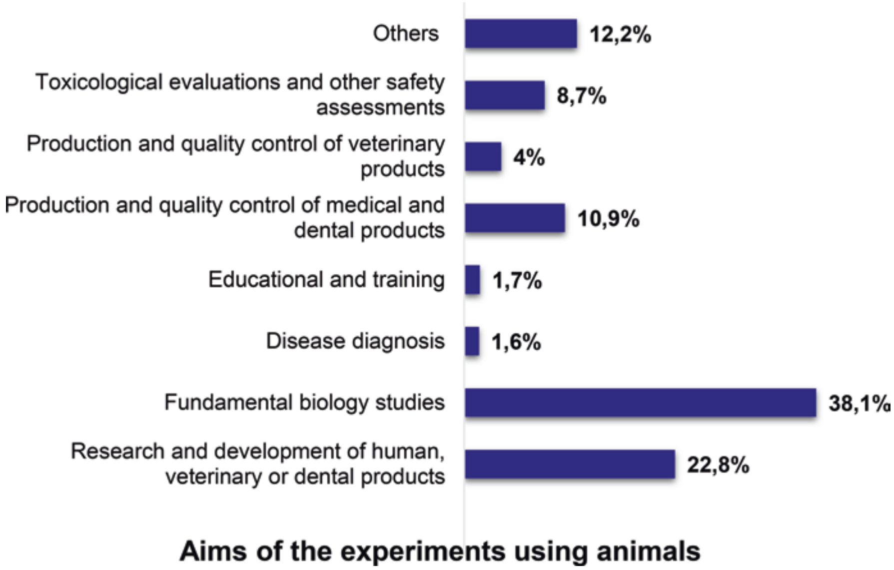

Fig. 10.1 Uses of animals for experimentation in the European Union. (Source: European Commission 2010)

world and provides guidelines for procedures when using animals in experiments (Demers et al. 2006). And this is where ethics in research come into play.

Ethics may help establish a basis for how animals should be treated and used in science frameworks (Carenzi and Verga 2009). Undoubtedly this also relates to ideas about animal welfare (Fraser 2008), as well as to the way we understand the principles of scientific ethics. Various studies have shown how the pressures to produce results can encourage misconduct in observing basic ethic norms in scientific research (De Vries et al. 2006). Kilkenny et al. (2009), for example, found that 4% of 271 journal articles the researchers reviewed did not report the number of animals used anywhere in the methods or results sections

The review process defined by the Institutional Animal Care and Use Committee (IACUC), an American system to oversee vertebrate animal use and ensure compliance with federal regulations and guidelines, has occasionally dealt with vested interests in animal experiments that have resulted in unnecessary animal use and suffering (Hansen et al. 2012).

Assuming that animal tests for different experimental procedures are necessary, two English biologists, Bill Russell and Rex Burch (2015) tried to do animal testing in an ethical way. Showing concern for animals' welfare, they suggest in their book, *The Principle of Humane Experimental Technique,* the '*Three Rs principle*' -- replacement, reduction and refinement. These two investigators proposed : *replacing* animal experimentation by other methods not involving their use (such as replacing animals with by *computer*-based *models* or by replacing vertebrate animals with invertebrate species, which may have less pain perception); *reducing* the number of animals used, when possible, through strategies that make more efficient statistical use of animal-based data; and by *refining* techniques used to minimize animal pain and distress while improving animal care and welfare, from birth to death (Nuffield Council on Bioethics 2005). Jharna Mandal and Subhash Chandra Parija (2013) believe that all researchers using experimental animals have a moral responsibility to the animals after their use, and suggest that rehabilitating experimental animals should be a legal requirement in India. These two authors proposed to add a fourth R, *rehabilitation*, related to the after-care and/or rehabilitation of animals post-experimentation.

10.3 Social Awareness Against Animal Abuse

Currently, social awareness of and resistance to animal abuse has grown exponentially, promoted by the Universal Declaration on Animal Welfare (UDAW), which was conceived in 1977 by the International League of Animal Rights and recognized by the Food and Agriculture Organization of the United Nations in 2009 (Haas 2014). In terms of scientific scope, students referring to themselves as *objectors of lab practices* involving animals intervention and sacrifice have helped reject previous animal-care practices and put into question continuation of such practices. They have initiated discussion about, and seek alternatives to, animal testing. But ethical considerations and arguments alone are unlikely to initiate movement or social change (Gruber and Hartung 2004): financial incentive usually is an essential driver for changing procedures in basic research to animal-free procedures.

We are against the use of animals in experiments. However, that option is not always possible. We expect few people would choose to use a new chemical compound, not previously tested either for efficacy or safety, unless it had been evaluated first using animals metabolically similar to humans. Respecting strictly the recommendations of the Committees of Ethics in Research, and following the 'good old strategy' of 4R, with adequate anesthetics and a compassion for other living beings, we suppose that research with animals could be compatible with methods used to avoid or reduce animal suffering. Let's be honest: by far most animals that are bred for human use are those that are used as food, and these animals likely are exposed to a level of suffering that is similar to, or higher than, that of lab animals.

But animals used for teaching pose another and a very different issue. Animal experiments are unnecessary in most colleges and careers. If teaching situations in which animals is absolutely necessary – for example for practicing a new chirurgical technique that requires (without known alternatives) the use of living animals – the number of experimental animals can be reduced by sharing them between experiments, or using videoconference systems to limit the number of animals used. Even in cases that absolutely require animals for teaching, we can improve the statistical designs so as to avoid the use of more animals than actually needed. Alternatively, or in addition to, teachers can sometimes replace animals with other animals lower on the phylogenetic scale, using "simpler" or "less sensitive" animals whenever possible (this could be discussed because we don't know yet how to measure animal suffering); refine the techniques that reduce animal pain; use techniques such as magnetic resonance imaging to minimize invasive procedures, as we do in human surgery; and so on.

Animal experiments are also used in large numbers in industry, particularly for producing confectionery, cosmetics and pharmaceutical products. In vitro assays can replace animal testing some or many cases, even as a progressively aware society demands cruelty-free products. Cruelty-free brands offer various cosmetics, personal care and household products that have not been assayed, or obtained from animals (https://www.crueltyfreeinternational.org/). Brands can be certified as cruelty-free, and their sales can benefit from such branding. However, fraud can occur at this step, as well, because the label per se may not warrant a product is actually animal-free. In at least a few cases, the labels do not accurately describe the product. For example, pork traces have been found in products labeled as apt for vegetarians (e.g., Muñoz-Colmenero et al. 2015). The good intentions therefore should be accompanied by appropriate systems to monitor and report such that consumers can reliably and accurately choose to buy what they intend to buy.

10.4 Animal-Free Teaching Alternatives

The goal to achieve should be to eliminate the animals from the experimental procedure in teaching practices. Let's think about current teaching practices still conducted in many genetics labs, worldwide. Experiments with fruit flies (*Drosophila melanogaster)* involve rearing and killing millions of flies, for the sake of teaching students principles of Mendelian inheritance, epistasis and the heritability of sex-linked traits. These principles could be easily learned from plants. Mendel inferred the inheritance laws from peas. But we don't need to go back in time, and we don't need to develop a botany lab, although it might well be welcomed by students. Science itself has progressed enough such that methods are available to reduce or avoid using animals in many teaching practices. Here are several specific examples. Alternatives-to-animals methods now include in vitro procedures for cultivating cells, tissues and organs; immunochemical techniques are available for cell, tissue and organ cultures; physical and/or mechanical systems are available for analyzing reactions in a defined set of predetermined circumstances; we have 3D representations of key anatomical features of animals, in plastic or other materials; and we have computer simulations, videos, and movies that capture, both in real time and in slow-motion, many functional operations of animals. Many areas of genetics, cytology, and toxicology also can be taught from a student's own cells and DNA (e.g., Pardiñas et al. 2010), and mucosa cells for obtaining DNA or for making cytological observations can be obtained simply rubbing the inner mouth with a cotton swab

Fig. 10.2 A genetics laboratory at the University of Oviedo

(Fig. 10.2). We suggest the primary limiting aspect of limiting or reducing animals use for teaching is that of teacher imagination (Dopico et al. 2014).

Other subjects, such as behavioral or field ecology, ornithology, aquaculture, or fisheries ecology, can depend on fieldwork, observations, and collecting and analyzing animals. How can students learn ecology, which can include animal sampling, without practice? Again, technology sometimes can help us. Animals leave traces of themselves in the space they inhabit: hairs, saliva, feces, footprints, fecal or urine deposits, and cells. A recent technique for isolating and analyzing DNA from water or soil might help in this regard. DNA from such sources can be referred to as *environmental DNA* (eDNA). From DNA sequences in the environment, investigators can identify the organisms that inhabit or which have passed through the environment. Such studies have been done now in different ecosystems (e.g., Taberlet et al. 2012). The method for sequencing eDNA is still relatively expensive, and analyzing eDNA sequences requires specialized bioinformatics skills (c.f. Zaiko et al. 2015). However, costs for these analyses are going down rapidly as the method becomes more popular, and we anticipate user-friendly software will soon be developed. Soon, it will not be necessary to disturb many fish to obtain a fish-species inventory for a pond; rather, the information can be obtained through eDNA analysis from a single one-liter water sample. If you want your students to observe animal behavior, underwater cameras and hydrophones can be positioned to record pond denizens (e.g., http://uw-observatory.loven.gu.se/about.shtml). Silent mini-robots with recording and data-reporting capabilities may be controlled by students and driven here and there within a pond, capturing animal images and sounds. If a species is extremely interesting, we don't need to kill and stuff a specimen for each college. Rather, replicates can be obtained by scanning and 3D printing, and the replicas can be distributed to the interested institutions. Although the current technique for doing this is expensive, new more affordable prototypes are being developed now and likely will become available soon (https://www.newscientist.com/article/dn7165-3d-printer-to-churn-out-copies-of-itself/).

Fine chemical analyses of the biosphere and virtual simulation and physical replication models can let teachers teach about nature without using or disturbing living animals. The challenges posed by rapid developments in biotechnology afford a hopeful track that may become generalized in the very near future. If the hopeful monster is a cruelty-free world, the hopeful monsterette could be science applied to cruelty-free methodologies.

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