The evolution of animal ethics regarding experiments in Europe from the late 20th century until now

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Abstract

Regulations regarding non-human animal (hereafter; animals) experiments have not been around for a long time. The last hundred years have seen the biggest changes in animal ethics and experiments. To understand what changed in the way humans handle and view different species, this review analysed the most important events of the last 70 years regarding animal welfare and animal experiments. Most experiments before the 21st century were performed on any type of species. Animals were mostly seen as machines, not able to feel pain and have emotions. Due to the rise of the anti-vivisection movement, as well as an increase in research on the physiology and behaviour of animals by different ethologists, the social and ethical demands for regulations in animal experiments increased. The 3R principles created by Russel and Burch in 1957, also played a key role. The principles of replacement, reduction, and refinement are up to this day an important part of the legislation regarding animal experiments. The concept of welfare became more developed and tangible, and it began to include the physiology, emotions, and adaptability of animals to assess their welfare. The most significant changes on international level happened when the EU directive 2010/63 was implemented. This directive levelled the playing field between countries, creating clear guidelines for the handling and care of animals in laboratories. Ethical committees became obligatory to approve experiments on animals. Furthermore, animals with higher cognition or abilities to feel more pain, and species that have cultural or social values to humans have been given a special status with stricter guidelines in the international legislation. This does emphasise that humans do not see every species as equal. The way humans handle and view other animals is predominantly based on the function that these animals have in the lives of humans. Alternatives such as computer models and cell cultures as replacements for animal experiments have been increasing as well. Still, the past years have also shown that animal experiments are still incredibly important when it comes to general science as well as health and medicines. It is therefore not expected that animal experiments will be banned anytime soon. For as long as animals are still necessary, humans have to do all they can to enhance the living conditions of laboratory animals, and always keep their welfare as the number one priority. Only if the number of animals used in experiments is reduced every year, can humans navigate towards a world without animal experiments.

Laymen's summary

For thousands of years, humans and animals lived side by side in a constant symbiotic relationship. As animals were essential for agriculture, food, fibre, locomotion and power, humans took good care of them and provided them with mostly optimal living conditions. When science and research became an important part of human societies, humans took it upon themselves to also use animals for this field. By studying the physiological features of different animals, more knowledge became available about the laws of nature, as well as the mechanisms and functions of the human body. In the first half of the 20th century, animals were widely used in a variety of experiments that were critical in the advances of the bio-medical field. Antibiotics, chemo and radiotherapy for cancer, the eradication of small pox, safe blood transfusions, as well as the discovery of DNA, are just a small part of all the discoveries made during this period with the use of animal experiments. With the rise of animal experiments, the social demand of better circumstances for these animals increased as well. The second half of the 20th century, as well as the first decades of the 21st century saw significant changes in the handling and care of animals in laboratories, as well as a change in the general idea of animal welfare. To understand what motivated these changes in Europe in such a short period of time, this review highlighted and discussed the most important contributing factors, laws, and people over the past 70 years. It furthermore discussed how humans have different opinions about different animal species. Ethology, a new field in biology, was on the rise in the second half of the 20th century. Ethologists and psychologists made an incredible difference in the human perception towards animals. Next to using animals for experiments that benefited mainly the medical advancement for humans, scientists were now beginning to study animals to understand more about their complex nature and behaviours. The concept of animal welfare became more tangible, and more countries and organisations became concerned with the welfare of agricultural as well as laboratorial animals. In the early 2000s, more scientists started to study intelligence and cognition in different animal species. Animals were not just merely machines anymore, as it was found that many different species actually have high cognitive abilities. In 2010, the European Union published a directive in which new rules became obligatory for all member states regarding animal

experiments. This included minimum standards for housing, care, treatment, as well as the use of welfare committees. The species with higher cognitive abilities and species that have cultural or social values to humans have been given even stricter guidelines. This emphasises that not every species is equal in human eyes. The way humans handle and view other species is predominantly based on the function that these animals have in the lives of humans. The last two decades saw a tremendous increase in alternative methods to reduce the number of animals in experiments. Nevertheless, animal experiments are still necessary when it comes to general science as well as health and medicines. Still, with the rise of new technology and better alternative methods, combined with the growing social demands for better welfare of animals, it is expected that things will continue to change for the better regarding animals in laboratories.

Introduction

Regulations regarding non-human animal (hereafter; animals) research have not been around for a long time. The last hundred years have seen the biggest changes with regard to social animal ethics, in which laws have been conducted which aim to safeguard animals during experiments (Franco, 2013). Before this, there were not many rules to be found. Only the Old Testament, which was written somewhere between 1500 BC and 400 BC, stated a prohibition against overt cruelty towards animals, as this was considered sadist (Rollin, 2006). For thousands of years, humans and animals lived side by side in a constant symbiosis. As animals were essential for agriculture, food, fibre, locomotion and power, humans took good care of them (Rollin, 2018). Animals provided for humans, so humans knew that it was vital to have good living conditions for their life stock. Mostly, it was not necessary to create rules for animal ethics as animals were treated well, and as far as known animal experiments were not yet performed. Physicians in ancient Greece were one of the first to use living as well as dead animals for medical studies, as human dissection was seen as a taboo (Baumans, 2004; von Staden, 1992). In general, it was not considered necessary to have some form of regulations regarding these experiments. The viewpoint during this time was that humans have the highest place in the *Scala Naturae* (the chain of being), created by Aristotle, and any other breathing and living organism was beneath them (Granger, 1985). Until recently, this vision has remained quite unchanged (Fortenbaugh, 1971).

Animals were continued to be used for experiments until the early middle ages in Europe. However, with the decline of the Roman empire came also the decline in scientific experiments and vivisection (Franco, 2013). During this time, in the early middle ages (5^{th} – 10^{th} century), the Christian church gained the ruling power and became far more controlling (Evans, 2007; Noble & Head, 2010). Science and religion did not go together, and the church banned all scientific research, including animal experiments. There was no reason to study the anatomy or physiology of animals and humans, because it would go against all the laws of religion. Furthermore, people were far more concerned with eternal life than biological laws and processes. Animal experiments would reappear during the Renaissance between the 14^{th} and 17^{th} century (Franco, 2013; Toledo-Pereyra, 2015). The Renaissance highlights a period wherein fields such as culture, arts, science, and politics were revived. The church started to lose some power, and there was more freedom to explore other topics in life besides faith. One of the key persons during this time was Francis Bacon (1561-1626). He believed that experimental encounters were the only way to test a hypothesis, and that only through experiments can the truth about nature be discovered (Hochberg, 1953). He became the founder of modern scientific methodology, and was a strong advocate of vivisection (Merchant, 2008). No laws and regulations were in place for these experiments. Another important person during the Renaissance was René Descartes, a famous French philosopher and scientist. In 1637, he wrote the book '*Discourse on the Method'*, where he stated that animals were organic machines, lacking language and intelligence (Descartes, 1637). In his opinion, animals did not have souls, thoughts, reasons and feeling, and can therefore not be categorized with humans (Rollin, 2006). This opinion stimulated the viewpoint that animal experiments were morally right, and further encouraged vivisection.

In the early 1800s, western societies created some regulations based on the Old Testament, called anti cruelty laws (Letourneau, 2003; Riley, 2022). However, these laws were not to safeguard animals' rights, but focussed on the indirect effect animal cruelty had on humans. If somebody would be cruel to another person's animal, this was seen as damaging another person's property and therefore considered a crime (Broom, 2011). Furthermore, there were concerns that if a person was capable of cruel acts towards animals, it would not take them much to also perform cruel acts to humans (Rollin, 2006). However, if by performing these cruel acts towards animals, it would keep the person in question from hurting other people, the sadistic acts were actually approved. Nevertheless, morale was changing during this century. People started to become sensitive to animal cruelty (Bardell, 2004; Wilson, 1997). A German philosopher named Richard David Precht suggested that because of the industrial revolution, animals became less important for the direct livelihood of people. There was less pressure on surviving, which created space for people to think about other issues, such as animal welfare and cruelty (Precht, 2011). In this century, there were also a few very important scientists and philosophers who changed the viewpoint on nature and biology. Charles Darwin (1809-1882) described the evolution theory, which put the religious viewpoint on life under a lot of pressure (De Ruyver, 2021). Through his research he created the 'Tree of Life', wherein he determined that humans share common ancestors

with other animals (Darwin, 1859). This theory completely changed all biological concepts. It also went against the '*Scala Naturae",* which had been the main argument for thousands of years. Humans were not the pinnacle of creation anymore (Campbell & Hodos, 1991; De Ruyver, 2021).

Based on the new insights, the United Kingdom introduced their first animal protection organisation, the Royal Society for the Prevention of Cruelty to Animals (RSPCA) in 1824 (Blazina *et al*., 2011). It was also the first country to introduce Cruelty to Animal Acts in 1835, which was updated again in 1849, 1876 and 1911. Other European countries followed, with the Netherlands being the last one in 1864 (Franco, 2013). These organisations were mostly concerned with the treatment of cattle and pets such as horses, cats, and dogs (Moss, 1961). They did not focus on animals used for scientific purposes. However, in 1976, the Cruelty to Animals Act of 1849 was amended by the British Parliament to include laboratorial animals (Finn & Stark, 2015). This act was established as a reaction to the public's opposition towards vivisection. The Act put down a set of rules for animal experiments, but only included vertebrate animals (Lane-Petter, 1962). For instance, it was prohibited to perform painful experiments on animals. However, if the painful experiment was absolutely necessary to save or prolong a human life, the experiment would be approved (Finn & Stark, 2015). Another important rule was that animals used in experiments should always be anesthetised and used only once. After the experiment was finished, the animal should be killed immediately (Balls, 1984). Furthermore, licenses and registration were needed in order to perform experiments (Lane-Petter, 1962). The implementation of this law was a very important step in a direction which focussed more on safeguarding animals' welfare. People started to accept that animals deserved to be treated with respect and must be protected from cruelty. In 1892, the British writer Henry Salt (1851-1939) wrote the book Animal's Rights: Considered in Relation to Social Progress (Salt, 1894). He was one of the first advocates for animal's rights, and pleaded against vivisection, fur trade, hunting and fishing. He stated that animals should be seen as persons, and not as things (De Ruyver, 2021). Furthermore, he argued that the idea of speciesism should be banned. His plea accelerated the animal rights movement.

In the first half of the 20th century, science developed rapidly. Not only did (bio)medical research in itself increased in popularity, new methods became available that made it easier to study the laws of nature. Advances were made in regards to medicine and curing diseases, mostly with help of animal experiments (Franco, 2013; Rollin, 2006). Antibiotics, vaccines, chemo and radiotherapy for cancer, the eradication of small pox, DNA, the discovery of insulin and hormones, as well as safe blood transfusions, are just a small part of all the discoveries made during this time period (Arruebo *et al*., 2011; Bristow *et al*., 2006; Henderson, 1987; Hutchings *et al*., 2019; Plotkin, 2005; Shander, 2004). Another important moment in biomedical research was the creation of the Wistar Rat (*Rattus novegicus*) in 1906, the fist standard rat strain used in laboratories. The house mouse (*Mus musculus*) was predominantly used in laboratory studies, but with the development of the Wistar rat strain, a new model organism was created (Clause, 1993). Furthermore, Lucien Cuénot continued with Mendel's inheritance theory and used mice in 1905 to demonstrate the concept of genes, which indeed followed the laws of Mendelian inheritance (Limoges, 1976; Paigen, 2003).

During these decades, the animal agriculture started to change to industrial agriculture. With this change, the husbandry circumstances of many agricultural animals started to decrease (Franco, 2013). The factories and farms were designed in such a way that they could house the highest number of animals in the least amount of space, with the lowest amounts of costs. This was beneficial for humans, but not for the animals. This sparked new social demands for animal ethics related to agriculture. Furthermore, funding for animal research increased, and more animals were used for e.g. toxicity testing (Rollin, 2006). This area of science was very damaging to the welfare of animals in laboratories. These factors contributed to the rapid increase of the anti-vivisection movement in the first decades of the 20th century, which was built on Salt's animal rights activism (Salt, 1894). However, this movement did not have any solid grounds to act upon as the previous named discoveries or changes were so vital, increasing for instance food supply and human life expectancy. Furthermore, during these decades the world had to deal with two great world wards, a huge economic recession as well as the start of the cold war (Rollin, 2006). As these events had such an impact on the whole world, the fight for animal rights and ethics was not that important to the people just yet.

Most experiments before the 21st century were performed on any type of animal. The ability to feel pain and emotions was not ascribed to animals, and it therefore did not matter which animals were used (Broom, 2011). In laboratories, rats and mice were very often used for experiments. Besides the

fact that they were very easy to handle, they were also seen as pests, making it morally acceptable. Still, animals from shelters, livestock and all different types of apes and monkeys were also regularly used. After the second world war, some governments even promoted the use of animals from shelters for experiments (Rollin, 2006). The anti-vivisection movement started to rise again after the war, but the biomedical research community fought against the allegations. Animal research was portrayed as a scientific necessity, and anyone against it would be labelled an "*animal lover and a people hater"* (Rollin, 2006, p. 288). The idea that there were ethical issues regarding animal experiments was ignored by many scientists.

Many centuries have passed since the start of animal experiments in ancient Greece. Much has happened, and even though some laws have been made regarding animal welfare and animal ethics, it seems that implanting changes have a slower rate than what might be necessary. At the end of the first half of the 20th century, the anti-vivisection movement stagnated and maybe even declined. In contrast, the second half of the 20th century actually saw great changes regarding animal experiments, animal welfare, and animal ethics. New laws appeared, and the philosophy of many people and scientists started to change. To understand what motivated these changes in Europe in such a short period of time, this review will highlight and discuss the most important contributing factors, laws, and people over the past decades of the 20th century up until the 21st century. It will be analysed how humans perceive different animal species and what motivates these views. Lastly, it will be discussed what the possible changes could be in the upcoming decades regarding animal experiments as well as animal ethics.

Changes throughout the 20th and 21st century

The first half of the 20th century saw many discoveries in science, including in the medical field. Many of these discoveries were related to animal research and of utmost importance to our understanding of different biological concepts and to our overall health systems. One way this is evident is in the amount of Nobel Prizes in physiology or medicine given since 1901. From 1901 until 2012, 83 Nobel Prize winners were scientists who worked on vertebrate species in their research (Franco, 2013). Besides the increase in (laboratory) animal experiments, there was also an increase in another field of science, namely ethology. Ethology is defined as the study of animal behaviour, especially under natural conditions. It is important to address that even though this review is focused on the changes in regulations surrounding animal experiments throughout the years, these changes are influenced by the different discoveries in fields such as ethology as well as agriculture, and will therefore also be discussed.

In the early 1950s, the consensus around animal experiments changed through research done by different ethologists and psychologist on motivation systems in animals. Konrad Lorenz (1903-1989) was a big advocate for studying animals' behaviour under natural circumstances instead of laboratory settings. Lorenz is best known for his work on imprinting and attachment in animals (Lorenz, 1950). In 1951, Nikolaas Tinbergen (1907-1988), a good friend of Lorenz, published his book '*The Study of Instinct'* (Tinbergen, 1951). This book focused on the innate behavioural reactions of animals. Tinbergen is also known for his 'four questions', which he stated are questions that should always be asked about animal behaviour (Tinbergen, 1963). These questions were as followed: 1) Why is the animal performing a particular behaviour (regarding function or adaption of the behaviour; 2) How did the behaviour evolve (regarding the evolution of phylogeny of the behaviour); 3) What causes the behaviour to be developed? (regarding the causation or mechanism behind the behaviour); and 4) How was the behaviour developed during the lifetime of the individual (regarding the development or ontogeny of the behaviour) (Bateson & Laland, 2013; Tinbergen, 1963). These questions highlight an important change in mentality when looking at animals and their behaviour. They dismiss the idea that animals are just robots, who have no control over their actions and/or behaviour. It challenges researchers to go more in depth to figure out the meaning behind behaviour. To this day, these four questions are still used to understand and describe animal behaviour.

Around the same time, Robert Hinde studies motivation systems, as well as behavioural development and social relationships in primates (Bateson *et al*., 2018; Hinde, 1954). Another important person in this decade was Neal Miller, a psychologist at Yale University. He conducted experiments on rats, where he discovered biofeedback mechanisms and advocated that humans (and possible other

animals too) are able to self-regulate autonomic responses (Miller, 1957). These ethologists, zoologists and psychologists made an incredible difference on the human perception towards animals. Next to using animals for experiments that increased mainly the medical advancement for humans, scientists were now beginning to study animals to understand more about their complex nature and behaviours. Even though Neal Miller was still a big advocate for animal experiments, and most of his work was done using laboratory animals, he also addressed the complex issues surrounding animal experiments. During this time, the discussion of animal ethics started to focus more on what people should do in regards of animal protection. The discussion about animal welfare, in terms of what an animal 'needs', such as optimal living conditions, was not present yet. This was partially due to the fact that studies regarding animal behaviour (ethology) were only just upcoming, and the knowledge to enhance animal welfare was still lacking. Moreover, the opposition from the biomedical community was still very strong.

In 1959, perhaps far ahead for their time, William M. S. Russel and Rex L. Burch created the Principles of Humane Experimental Technique for the Universities Federation for Animal Welfare (UFAW) (Russel & Burch, 1959). Most people in the bioscience field know these principles as the Three Rs (Russel, 1957). The principles were created to enhance the welfare of vertebrate animals used in experiments, and was a collaboration between the UFAW and the scientific community (Hubrecht & Carter, 2019). This is in itself remarkable, as welfare organisations and scientists did no go hand in hand during that time (Russell, 1995). The three Rs were defined as:

- Replacement: The substitution for conscious living higher animal of insentient material (Russel & Burch, 1959, p. 69);
- Reduction: Reduction in the number of animals used to obtain information of given amount and precision (Russel & Burch, 1959, p. 105);
- Refinement: Any decrease in the severity of inhumane procedures applied to those animals, which still have to be used (Russel & Burch, 1959, p. 134).

Even though the field of ethology was only just upcoming, Russel and Burch highlighted the fact that animals could indeed have consciousness and that the stress experienced by animals in research could influence the experiments and bias the data (Russel & Burch, 1959). Furthermore, they provided good examples for humane methods in research. Nevertheless, the concept was quite new, animal welfare was still not well understood, and due to some negative reactions towards it, the three R's principles only started to become more implemented a few decades later in the early 1980s (Hubrecht & Carter, 2019).

The dawn of a new era

The decade between 1960 and 1970 saw some of the most significant changes with regard to animal welfare and experiments. In 1964, Ruth Harrison, an English welfare activist, wrote the book 'Animal Machines'. Here, she advocated the rights of farm animals as she stated that the animals used in the agricultural industry were very often treated as inanimate machines, rather than living individuals (Harrison, 1964). Not only does she emphasize the importance of animal welfare, she also touched down on the topic of the moral injustice that was done to these animals. Scientific research and food production industry had to be morally justified, and animals should be treated humanely. When this did not occur, it would diminish the humanity of humans who allowed such treatment to be legal (Kirchhelle, 2021). Her biggest strength, and also what made her book so important, was the fact that she understood a frontal attack would only elicit a defensive response. Instead, she focussed on the personal aspect of animal treatment, namely the humanity of people (Harrison, 1964).

In the same year, the British Brambell Commission came into existence, partly as a response to the allegations Harrison made in her book regarding the agricultural sector (Broom, 2011). This committee, led by professor Roger Brambell, investigated the welfare of intensely farmed animals. Through these investigations, it was determined that to have morally justifiable agricultural systems, the basic needs and nature of a particular animal should be respected, especially when they are being used by humans (Brambell, 1965; Mench, 1998). The Brambell Commission released a report wherein it was emphasized that every animal has biological needs for which it is essential to show specific behaviours. When these behaviours could not be expressed due to human treatment or housing conditions, the welfare of the animal would decrease (Brambell, 1965). This was incorporated into the

"Five Freedoms", in which an animal must have 1) freedom from hunger or thirst; 2) freedom from discomfort; 3) freedom from pain, injury or disease; 4) freedom to express normal behaviour; 5) freedom from fear and distress (Brambell 1965). These five freedoms are regularly revised and updated (Ohl and van der Staay, 2012; Arndt *et al*., 2022;). Because of Ruth Harrison and the Brambell Commission, the ideology of welfare began to change as well, and the term became somewhat more defined. It was not just about the protection of animals, but more about what would be the optimal conditions an animal should be housed in, in order for that individual to express its natural behaviour. Of course, the ideologies were different than how we would determine welfare today, but that is due to the fact that research on animals' cognitive abilities was in its early days.

During this period, it became evident that there were two very different, extreme sides in the discussion on animal welfare and animal experiments (Rollin, 2006). One side consisted of a considerable number of members of the research community that found it unacceptable to suddenly have constraints on their work and use of animals in research (Visscher, 1982). Most of these people were raised and educated with the long-standing idea that anyone who raised any ethical questions regarding animal experiments were seen as anti-science, anti-human, and anti-progress (Rollin, 2006). The other side was that of the animal rights advocates who wanted to ban animal research altogether. The different viewpoints of these sides also highlight an important factor regarding change in humans. Humans are known to be creatures of habit, and behavioural changes take time and effort. For thousands of years, the overall mindset was that using animals for experiments was justified and important. It has become integrated as part of human culture. Such a fixed mindset is not changed within a day, even though there are many studies and reasons why it perhaps should be changed. Therefore, most people involved in animal research were probably not even against better guidelines surrounding animal experiments, but felt uncomfortable as they suddenly had to change their 'traditions'.

This also underlines the reasons why concepts such as the Three Rs only started to affect the attitude of people many decades after it was first published. The two sides (anti-vivisection movement vs the pro animal experiments) stood completely opposed to each other, creating a so-called no-man's land between them. However, in discussions about animal welfare and animal experiments (and discussions in general), it has never worked to favour one side over the other. In order to incorporate the necessary changes, the two sides have to find common ground. For instance, instead of focussing on abolishing animal experiments completely, it worked better to create better living conditions for animals and find replacement alternatives for the experiments (Balls, 1995).

In 1975, Peter Singer, an Australian moral philosopher who is specialized in applied ethics, published his book 'Animal Liberation' (Singer, 1996). He was inspired by both Ruth Harrison and Henry Salt. Singer defined the principles of speciesism which has many similarities with the '*Scala Naturae'* principle (Granger, 1985). For as long as can be remembered, humans have been prone to attribute a low moral value to animals, on the sole basis of belonging to a different species. He argued that just as racism or sexism is unjustifiable and completely wrong, so is speciesism. Humans differ in levels of intelligence. Some people are born with much higher IQs, while others might be less fortunate in that department. Nonetheless, this difference does not indicate that humans possessing higher degrees of intelligences are entitled to exploit other humans with less intelligence, or use them for their own ends. Therefore, Singer proposed that animal rights should not be based on the level of intelligence of a species, but on their abilities to feel pain (Singer, 1996; Singer 2009). Singer stood up for animal rights in a variety of ways. He criticized the meat industry and drew attention to the welfare of fish, something that was often forgotten. In his book, he furthermore criticized animal experiments. Humans inflict a great amount of suffering to animals used in scientific experiments, and these animals deserve to be treated with respect considering their ability to suffer and feel pain. Nevertheless, he did not take an absolute approach where he wanted to ban all animal experiments. If by testing on animals a cure would be found for an otherwise incurable disease, the experiment could be justified, according to Singer (Foëx, 2007).

During the 1970s, the Three Rs principles regained new attention. As the number of animals used in laboratory experiments peaked, the alternative approach towards animal experiments was brought under the attention by many animal protection movements (Hubrecht & Carter, 2019). A few countries in Europe such as Denmark, Switzerland and the Federal Republic of Germany created laws focussed on animal research and support for alternatives. In 1971, Sweden became the first country in which government funding was provided for scientists who developed alternatives for animal

experiments (Svärd & Tinnerholm Ljungberg, 2021). The Dutch government followed shortly hereafter, and established the Experiments on Animals Act in 1977 (Stephens *et al*., 2001). Again, funding was provided for alternative research. In 1981, the Dutch government furthermore supported the assumption that the intrinsic value of an animal should be the '*explicit point of departure for its policy on human-animal relationship'* (Brom & Schroten, 1993, p. 100). It became evident that animal experiments have conflicting interests for humans and animals, and that the pros and cons for both sides should be weighed up against one another. The Dutch government started to recognize animals as moral beings, and wanted to preserve their interests in not only experiments but also in other animal uses such as agriculture. In 1983, Utrecht University in the Netherlands began to create programs on animal research and education, with the focus on further implementation of the Three Rs. Three years later, the Council of Environmental Ministers of the European Community instructed all member countries to develop laws which promoted the principles of the Three Rs (Svärd & Tinnerholm Ljungberg, 2021). Initially it was animal rights activists and animal protection groups who heeded for laws and regulations in animal research, but this period highlights the switch in which governments started to actually legislate.

At the end of the 1980s, the Dutch government started to create rules in which the protection of animals became the central viewpoint. In the Draft Animal Health and Welfare Act, it was stated that animals are not to be used in experiments, *unless* the Minister of Agriculture, Nature Management and Fisheries gave consent (Brom & Schroten, 1993). Plans for animal experiments first needed to pass an independent committee, consisting of experts who were appointed to give their advice surrounding ethical questions and challenges. The principles of these committee were *autonomy, beneficence, non-maleficence, and distributive justice relationship'* (Beauchamp & Childress, 1979). Animals used in experimental setting need to be respected in and for their natural and intrinsic values, and their housing conditions and welfare needs to be optimized. Furthermore, the non-maleficence principle indicates that no harm should be done to animals, and if harm is necessary in experimental settings, it needs to be justified. Lastly, the end should justify the means, meaning that harm done to the animal should equal the benefits of the experiment to humans. The Animal Health and Welfare Act was approved by the Dutch government in 1992, and in the same year another committee was installed; the Provisional Committee for Ethical Assessment of Genetic Modification of Animals (Vorstenbosch, 1993). As technology began to develop more, transgenesis in animals began to increase as well. This was an area of science where there were not many rules and regulations regarding animal welfare and ethics, due to it being so new. Genetic modification in animals was (and still is) a very promising new field, but it also comes with many ethical questions. How much do humans see themselves as a 'God', if we use our knowledge and science to genetically change the structure and lives of animals? It was therefore of great importance that an independent committee should be in place to regulate all rules and regulations surrounding transgenesis and genetic modification.

Animal welfare

Around the same time, the concept of animal welfare began to change. Instead of it being an elusive concept, it became measurable and scientific. Furthermore, new models were designed to assess the welfare of animals. Based on the Five Freedom concept formed by the Brambell Commission in 1965, a new concept was formulated in 1994 called the Five Domains, which was specifically designed to assess the impact of experiments on the welfare of experimental animals (Mellor & Reid, 1994). The Five Domains consisted of 1) thirst/hunger/malnutrition; 2) environmental challenges; 3) disease/injury/functional impairment; 4) behavioural/interactive restriction; and 5) anxiety/fear/pain/distress. One of the biggest changes between the five domains concept and the five freedoms concept is how animal welfare is defined and analysed. The Five Freedoms was created to assess the welfare of farm animals, wherein it measured how 'good' the welfare of a certain animal is. The Five Domains focusses on experimental animals, where it does not focus on how good the welfare is, but how much of their welfare is actually compromised when partaking in experiments. There are some cases in which animals are used for experiments and almost no harm is done to the animal, however, these cases are rare.

The Five Domains concept is based on new welfare indicators regarding the physiology of the animal. The stress response, for instance, became an important physiological welfare indicator (Pohlin *et al*., 2021). When the adrenocortical activity of an animal increased, it could indicate a decrease in

welfare as the animal experienced more stress (Barnett & Hemsworth, 1990). Another import welfare indicator was found on the behavioural side of animals. As more and more behaviour of species was analysed, changes in behaviour became more understood. If abnormal behaviours were seen, such as stereotypes, it could also indicate a decrease in welfare (Broom, 1983). It is important to note that change in itself, whether this is physiological or behavioural, is not bad per se. Every living organism on the planet wants to obtain homeostasis. In order to accomplish homeostasis, an organism needs to be able to adapt to changing environmental and physiological circumstances (Barnett & Hemsworth, 1990). Therefore, the change in itself is not detrimental. Is about the direction and the magnitude of change (Barnett & Hemsworth, 1990). Only by understanding this, it can be assessed at what level of change the welfare of animals is at risk. Stress is a good example for this. Acute stress responses are very different from chronic stress responses. Acute stress responses are essential for animals to survive and adapt to different factors. However, chronic stress responses bring about many long-term risk factors which are aversive to the animal (Barnett & Hemsworth, 1990). An increase in adrenocortical activity, as well as the appearance of stereotypes in animals, are mechanisms with which an animal can cope long-term with their changing environmental factors and can indicate a decreased welfare. Still, acute stress responses caused by harmful experiments should always be considered in indicating a decrease in welfare as well.

New century, new concerns

In the early 2000s, something shifted in human-animal relationships. Before this time, the basis of these relationships was mostly for agricultural reasons and food. This started to change into a utility and companion relationship. Farmers would still have large amount of livestock, but the regular civilian mostly had animals, e.g., dogs and cats, for companion. Furthermore, awareness for the loss of biodiversity started to increase. People started to realise what the impact of the human population was on ecosystems all around the world. It became clear that while the human population increased enormously, the amount of species started to decline (Moranta *et al*., 2022). One of the biggest factors contributing to this switch in awareness was the fact that epi- and pandemics started to become much more frequent and detrimental all around the world (Webster *et al*., 2005). It started with the avian flu epidemics in 2003, which still kills more than million birds every year all around the world (Aldhous & Tomlin, 2005; European Food Safety Authority *et al*., 2021). Furthermore, other infectious diseases such as severe acute respiratory syndrome (SARS), become much more severe and started to affect humans as well (Peter *et al*., 2013). These events changed peoples' mindset. One of the reasons that the avian flu epidemic occurred, and is still now a huge problem, is because of the man-made ecosystems created to house animals for production. Chickens, for instance, are mostly held in battery cages, meaning that thousands of chickens live side by side. This makes it very easy for infectious diseases to spread and wipe out entire farms of chickens. The avian flu caused many birds used in the poultry and eggs industry to die, which in turn decreased human food resources. Furthermore, wild birds can also become infected and die (Shriner *et al*., 2016). The SARS epidemics are transferred from animals to humans. This transmission also often occurs in places where a lot of animals are kept in a small space, with humans living close to them. The rise and spread of infectious diseases originating from animals highlights that how humans use and house animals not only endangers the food industry, but also has great impact on human health and ecosystem health.

Another major shift in human perception towards animals came through research that focussed on intelligence in animals. For most of the 20th century, the main idea was that only humans could perform higher-order cognitive tasks (Anderson & Gallup, 2015; de Waal, 2016; Morgan, 1929; Thorpe, 1957). Animals were mostly seen as robots, and therefore research on the inner life of animals seemed unnecessary. In 1970, Gordon Gallup had designed the mark-test, also known as the mirror-test, in which animals where tested to determine whether they were able to recognize themselves in a mirror (Gallup, 1970). The first tests were done with chimpanzees and macaques, in which only the chimpanzees passed the test. After that, many primate species were tested on self-recognition. Only chimpanzees, orangutans and bonobos passed the test (Hyatt & Hopkins, 1994; Lethmate & Dücker, 1973; Walraven *et al.*, 1995; Westergaard & Hyatt, 1994). However, at the beginning of the 21st century, other species beside primates were subjected to the mark-test. In 2002, two bottlenose dolphins were tested and actually passed the experiment (Reiss & Marino, 2001). Later, Asian elephants (Plotnik *et* al., 2006) and magpies (Prior *et al*., 2008) also passed the mark test. These results were fundamental in

understanding that cognitive abilities could not only be ascribed to members of the family Hominidae, but exist outside this evolutionary lineage in far more distant species. This increased research into the inner life and emotional and cognitive states of animals. It was furthermore found that corvids have an enormous encephalization quotient, equal to that of chimpanzees (Seed *et al*., 2009). Corvids also have many cognitive tools similar to that of great apes (Emery & Clayton, 2004). Another research found that fish could feel pain (Sneddon *et al*., 2003). All these studies showed that animals are far more intelligent than what was formerly assumed.

Levelling the playing field

The studies described in the previous paragraph provided new scientific knowledge regarding whether or not animals feel stress and pain inflicted by humans, and opened up new discussions surrounding animal welfare and animal experiments. In the first years of the 21st century, the European Consensus Platform for 3R Alternatives to Animal Experimentation, ECOPA, was established (Rogiers, 2004). This organization was formed to promote the Three R's on European level. All parties concerning animal experiments, animal welfare, industry, academia, and governmental institutions, are represented in this organization (Rovida *et al*., 2018). The main focus of this organisation was to raise awareness for alternative methods in animal experiments, on all possible levels (governmental, scientific and public) (Rogiers, 2004).

With European and international focus on animal experiments and the interconnectedness of human, animal, and ecosystem health, things started to shift. In 2010, the European Union published the Directive 2010/63/EU, concerning the protection and welfare of animals used for scientific purposes (European Union, 2010). This Directive was a follow up of the Directive 86/609/EEC established by the European Union in 1986, in which early international guidelines were created for the use of animals in research, including minimum standards for housing, care, treatment, as well as requirements for animal welfare committees and ethical review processes (EEC, 1986; Louhimies, 2002). The new directive from 2010 focused on many different aspects. It expanded the general focus of ECOPA and the Directive 86/609/EEC, to implement the 3Rs on an international level (Neuhaus *et al*., 2022). It not only states that mammals used in science should be protected, but expanded that protection to foetal forms of mammals as well as cephalopods, as research has shown that these species and early life forms experience pain and suffering (Smith *et al*., 2013). It emphasised that the use of live animals continues to be important and necessary, however, its ultimate goal is to find full replacement for all procedures in science where animals are still used (Olsson *et al*., 2017). It states that animals have intrinsic values which must be respected, and that animals should only be used for scientific experiments if there is no non-animal alternative available. For every animal experiment, the minimum number of animals should be used that would still provide reliable and unbiased results. The scientific benefits of experiments should weigh up against the suffering that animals experience (Eggel & Grimm, 2018). This directive was, and still is, one of the strictest standards regarding animal ethics and welfare worldwide. One of the main purposes of this directive was to create more uniformity among Member States in their laws and regulations regarding the protection of animals used in experiments.

The new regulations became obligatory for all members of the EU, and had to be implemented from the first of January 2013. It was an important turning point, as it levelled the playing field among countries regarding animal welfare. However, there are still many differences between countries (Guillén *et al*., 2015). Every Member State has to report every year how much animals they use and for what type of experiments. These reports include every animal, used either in experiments, killed for supplying tissue for *ex vivo* or *in vitro*, non-genetically modified and genetically modified animals used to sustain colonies, or other animals that are bred for scientific purposes but never used (Taylor & Alvarez, 2019). Furthermore, every Member State had to set up their own national organisations for review and authorization of the directive. There was, however, no direct guidance in how these committees should have been organised and which different parties should be involved when making decisions about animal experiments (Olsson *et al*., 2017). A committee with almost only scientists who experiment on animals will have different viewpoints on experiments than a committee filled with professors specialised in animal ethics. As these committees are therefore highly variable between countries, the impact that this directive has on animal welfare still differs across EU countries (Eggel & Grimm, 2018).

Nevertheless, this directive changed the view on how scientists handle animals and animal experiments. The Dutch government, for instance, stated that the Netherlands should aim to become the world leader in alternative experiments by 2025. In 2017, they created the organisation *Transitie Proefdiervrije Innovaties* (TPI). The mission of this organisation is to enhance animal-free innovations. However, the organisation realised that the deadline of 2025 to have all animal experiments banned in the Netherlands was a little too ambitious, and has therefore changed it goals. It wants to be a catalyst for the international transition to animal-free experiments (Baumgartl-Simons & Hohensee, 2019; van Veen, 2021). What also impacted the organisations surrounding the replacement of animal experiments, and animal experiments in general, was COVID-19. This pandemic highlighted that animal experiments are still necessary to ensure human health. All studies regarding the vaccines for COVID-19 made use of animals to test the impact, functioning, and safety of the vaccines (Kalnin *et al*., 2021; Kostoff *et al*., 2020; Lurie *et al*., 2020). If COVID-19 had struck after banning animal experiments all together, the pandemic would probably still be going on.

The countries in Europe have come a long way since the middle of the 20th century. Animals were first seen as robots, incapable of feeling and thinking. Through research on the physiology and behaviours of animals, much more is now known about the innate behaviours of animals and their ability to experience emotions and feelings. This is however, also the paradox of this topic. If there were no animal experiments, whether they were for the enhancement of human health or for understanding animals, we would not be in the same place we are today with all the information that is available. It seems that the use of animals for experiments has always been inevitable in order for science to grow. However, now that there is so much knowledge available, governments and scientists are unable to turn a blind eye towards the ethical questions regarding animal experimentation. Animal welfare movements continue to grow, and replacements for animal experiments have started to increase in reliability and usage.

Different species, different ethical questions

One of the most important shifts in the human viewpoint towards animals came from understanding animal intelligence and cognition. Through this type of research, it became evident that animals possess many abilities that humans had once thought were only applicable to humans. This also opened up the discussion about which animals are ethically okay to use for what type of experiments. From the beginning of animal experiments, no real consensus was made between species. Different animals were used, based on the availability and type of research. However, throughout the 20th century and the beginning of the 21st century, viewpoints, knowledge, and legislation started to change, also creating changes in what species can be used for what type of experiments. This part will highlight what these changes were regarding the different species used in experiments, as well as how new legislation has changed science.

The animals that have been used for experimental purposes range from non-sentient species like protozoa, to human's closest ancestors, the great apes. In the 20th century, countries mostly had specific regulations based on their own legal system (Franco, 2013). Mice, rats, guinea pigs, and other rodents, have been frequently used for experimental purposes in the 20th century, and still continue to be used often (Baumans, 2016). In the early decades of this century, rodents became one of the model species for animal experimentation (Wolff, 2003). This was due to multiple reasons. Firstly, rodents are easy to house and breed, as they have fast reproduction rates. Furthermore, they do not require extensive care or large housing facilities (Baumans, 2016). Secondly, rodents (especially mice and rats) have always been seen as pests (Stenseth *et al*., 2003). Lay people as well as scientists viewed these species as 'despicable creatures', therefore making it generally acceptable for research. Thirdly, rodents are extremely good model species (Ramsden, 2011; Vandamme, 2014). Before the EU directive in 2010, there were not many ethical considerations and regulations for rodent species used in research. As they are seen as species with a lower phylogenetic order and a lower moral value compared to other species, guidelines were never specifically put into place. With the new EU legislation, there are more rules that need to be followed when using these species in research. Minimum enclosure size and height, as well as floor area per animal, are included in the legislation (European Union, 2010). Furthermore, death

should be avoided as much as possible. If death is inevitable, there are rules on how the animal should be killed, experiencing the least amount of pain.

Fish have been used in scientific experiments for many years, but their importance in scientific research has increased over time as scientists have recognized their value as a model organism for studying a range of biological processes, as well as cognitive processes (Dunham, 2011; Egerton, 1987). Today, fish are one of the most used species in laboratories, including studies of neuroscience, behaviour, and diseases. This is because (small) fish are much cheaper to house and breed than many other species, and they take up less space. The zebrafish, in particular, has become a popular model organism for scientific research due to its small size, rapid development, and genetic tractability (Dahm & Geisler, 2006). In the EU directive, the stocking density and environmental complexity, as well as many other factors surrounding optimal welfare of fish, are included. Nevertheless, a very important debate in the use of fish for scientific purposes is whether or not they are able to experience pain. While fish lack the complex nervous systems of mammals, they do have a nervous system and brain structures that are capable of processing sensory information, including information related to pain (Rose *et al.*, 2014). Several studies have suggested that fish may experience pain in a way that is similar to other vertebrates (Braithwaite & Droege, 2016; Sneddon, 2020). For example, fish have been observed to exhibit behaviours such as rubbing or shaking an injured body part, avoiding painful stimuli, and exhibiting changes in behaviour and physiology in response to noxious stimuli (Sneddon *et al.*, 2014; Sneddon, 2015). The interpretation of these behaviours, and the extent to which they reflect the subjective experience of pain remains a matter of scientific debate. However, there is growing recognition of the need to ensure that fish are treated humanely and that their welfare is protected in scientific experiments and other contexts, which has resulted in more strict guidelines across the world (Braithwaite & Huntingford, 2004).

Animals that were frequently found in laboratories in the 20th century were cats and dogs. Both species were often taken from shelters or just picked up from the streets and sold to laboratories (Franco, 2013). As the demand for dogs and cats as companion animals grew intensively over the decades in the 20th century, people started to protest against stealing the animals from people's homes for experiments. Up until now, the use of cats and dogs in research is not illegal in Europe (Patterson *et al.*, 2020). However, under law by the European Union, the use of cats and dogs are very strictly regulated since the directive. Cats and dogs have (indirectly) been given a special status due to social and cultural perspectives, as they are mostly used as companion animals and have an important value to people (Directive 2010/63/EU, Art. 31). The EU only allows the use of these animals in exceptional cases where there are no alternative methods available and the potential benefits to human (and animal) health outweigh the harm caused to the animals.

Primates, including monkeys and great apes have been used in scientific experiments since the early 20th century (Kolar, 2006). Without a doubt, primates, especially great apes, are one of the best model species to be used in research. The use of primates is probably one of the most controversial topics across the world due to many ethical concerns. The more knowledge available about primates, the more ethical concerns arose about their welfare and the potential for harm. Several countries have now banned, or severely restricted, the use of great apes in scientific experiments. Under the EU's Directive 2010/63/EU, Art 55(2), experiments on chimpanzees, bonobos, gorillas, and orangutans have been officially banned for all member states (European Union, 2010). Other countries that have banned this type of research are New Zealand and Australia (Knight, 2008). Chimpanzees, orangutans, bonobos and gorillas can still be used in most countries for non-invasive observational research. This type of research is always aimed at improving their welfare and understanding their behaviour, and no invasive procedures or harmful interventions are involved.

Monkeys were and still are frequently used in laboratories. The segregation between monkeys and great apes in laboratorial conditions comes from the fact that monkeys are considered to have less cognitive abilities than great apes (Amici *et al.*, 2010; Parker & Gibson, 1977). Therefore, their use in research is believed to be more justifiable. Furthermore, they are easier to breed and maintain in captivity than great apes. The use of monkeys in scientific experiments is allowed under EU regulation, however, monkeys have also received a special status in the directive with more stringent rules that need to be adhered, concerning their housing and handling. Enclosures must be built in such a way that the animal is able to perform as wide a behavioural repertoire as possible. They can furthermore only be killed with anaesthetic overdose, which is considered the least painful and most humane way

(European Union, 2010). Monkey research is considered to be a last resort. When there are no alternatives available, and the potential benefits to human and animal health outweigh the harm caused to the animals, the use of monkeys is justified. Many experiments that were previously done with monkeys, such as polio vaccine testing, have been replaced with other species such as mice. Nevertheless, monkeys, mainly macaques and marmosets, are still frequently used in medicinal studies (Dijkman *et al.*, 2019; Sharpe *et al.*, 2010). Before any new medicine or vaccine gets brought out on the market, it has to be tested for safety and effectiveness on monkeys (Shedlock *et al.*, 2009).

There have been a lot of changes in the past few decades, and most species used in experiments are regulated by the Directive 2010/63/EU in order to protect their welfare. Animals cannot be used in laboratories without justification and the approval from the ethical committees. Researchers are expected to take good care of the housed animals, and provide them with the best life under these circumstances. Furthermore, replacement experiments are a key factor in this directive, motivating researchers to find alternative ways instead of testing on animals. There is however, still a clear divide between different species. As can be seen from figure 1 (adopted from Baumans, 2004), mice and rat are the most used species in laboratories. Guinea pigs, rabbits, birds and fish are also frequently used. Even though this graph is about twenty years old, it does highlight the trend in scientific research. Animals that are easy to house and breed, with a lower moral or cultural value and often seen as pests, will continue to be used the most in research. About 50% of the species mentioned in this graph experience moderate to severe discomfort during experiments (Baumans, 2004). It is believed that many of these species do not experience the same levels of pain as other higher phylogenetic species would. However, even with pain assessment models available for these species, these types of measurements have not been widely implemented in bio-medical research (Mota-Rojas *et al.*, 2020). Vital information on the animal's discomfort during experiments is therefore overlooked.

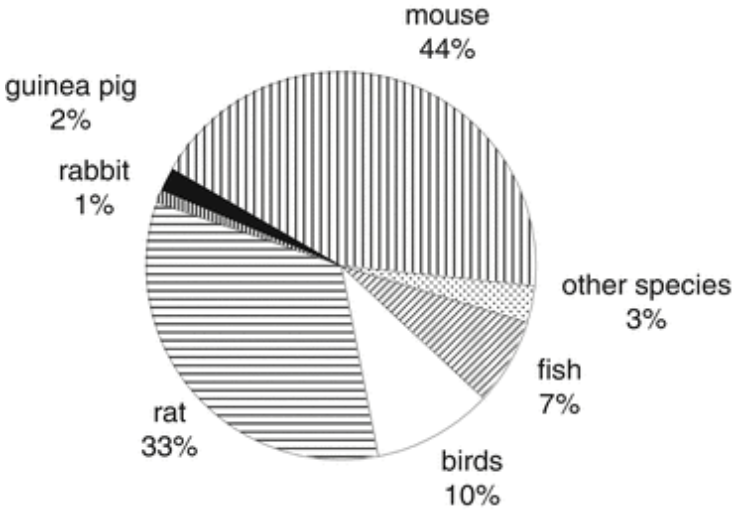

Figure 1. *Distribution of vertebrate animal species used for research, testing, and education (Adapted from Baumans, 2004, p. 10).*

The laws regarding higher phylogenetic species with a higher moral value for humans, such as great apes, monkeys, cats and dogs, have become much stricter. Besides the fact that many of these species do indeed have a higher cognitive level and can experience more pain, there are also a cultural and social aspects to these changes. Non-human primates are the closest living relatives of humans, and testing on primates comes with (more) emotional aspects due to their resemblance to humans (van Veen, 2021). Dogs and cats have also received a special status because of their relationship to humans. As stated in the Directive 2010/63/EU; only when no alternative methods are available, or the objective of the procedure cannot be achieved by using another species of a *lower* phylogenetic order, or when the benefits for humans outweigh the harm done to the animals, can these types of species be used (European Union, 2010). This scale, on which the benefits for humans and the harm to animals are put at opposite sides, is a difficult measurement. Not only does the scale change depending on the species, it also depends in which time the scale is set. In times of crisis, such as pandemics, the weight of human

benefit is much heavier than the weight of the harm done to animals. Until now, the only species that have been banned from (harmful) experiments are great apes (European Union, 2010). Besides that, most other species can still be used in laboratories.

Alternative methods

In recent years, science is gradually moving towards alternative methods instead of using live animals in research (Doke & Dhawale, 2015). This includes the use of computer models, cell cultures, simulated biological systems, and micro-dosing in human volunteers. Technological advancements have also played a significant role in the evolution of animal experimentation and alternative methods. Non-invasive imagining techniques, such as MRI and PET scans, have reduced the need for invasive procedures on animals (Wu & Miller, 2017). Furthermore, the use of genetically modified animals, particularly mice, has expanded the scope of research and allowed for more precise and targeted studies, therefore minimizing the number of animals used (Donehower & Lozano, 2009; Walrath *et al*., 2010).

Nevertheless, these alternatives come with some major challenges and ethical concerns (Adolphe, 1995; Fontana *et al*., 2021). First of all, alternatives lack the biological complexity that a whole organism has (Danku *et al.,* 2022). Computer models, cell cultures, and simulated biological systems do not provide the complexity of a whole living being. They cannot fully replicate the intricacies of biological processes, metabolic pathways, and interactions between tissues and organs in a living animal (Brandon *et al*., 2003; Griesinger *et al*., 2016). Important information could therefore be lost, or not provided by the alternatives. Another problem is with modelling complex interactions. Testing a drug or chemical on an animal will provide information on the interactions between the nervous system, hormones, and the immune system (Akkermans *et al.,* 2020). Systemic effects are not possible to study in isolated cells, tissues or organs (Adler *et al*., 2011). Bodies are designed in such a way that everything is connected with each other. Circulation, metabolism and excretion are all influenced by one another, and the effects of certain drugs on these connected systems can only be studied in living animals. Micro-dosing in humans provide more accurate and reliable data, as they are directly relevant to humans (Lappin *et al.,* 2013). However, the ethical guidelines surrounding micro-dosing on humans as an alternative method are difficult. This is because the concept in itself brings about much concern regarding the safekeeping of human volunteers. Furthermore, despite using humans in early trials, the chemical or drug itself first needs to be tested on animals before it will be injected into humans (Burt *et al*., 2016). Animals are therefore still necessary.

Another problem with alternatives is that many regulatory agencies still require the testing of drugs on live animals before approving (Akkermans *et al*., 2020). Alternative methods alone are often not considered persuasive enough for regulatory approval and licensing of new medical interventions (Adler *et al*., 2011). So, while alternative methods are promising and help replace some live animal research, it also comes with many challenges and concerns. Overcoming these challenges is key to eliminating the dependence on animals altogether and transitioning to an animal free scientific method.

Transgenesis and embryo use

An area that has been on the rise is the use of embryos and genetically modified strains of species, specifically bred for laboratory use. Some might say that embryos can be seen as alternatives to animal testing, as not a complete animal is used but only a small part. However, embryo use and transgenesis does not lead to less animal experiments in general. It should therefore be seen as another field of animal experiments that provides a lot of new and valuable information. Nevertheless, new ethical discussions on how rules apply to these organisms and/or cells are on the rise as well. In the last years of the 20th century, the use of transgenesis in mice and rat, as well as creating knock-out-genes, has increased tremendously (Charreau *et al*., 1996; Mullins *et al.,* 1990; Mullins & Mullins, 1996; Page *et al.*, 1995; Taurog *et al*., 1994). These types of experiments provide much information about the function and work of different genes. It furthermore helps humans understand more about genetic diseases and inheritance. However, the knowledge gained from these new research fields does not come without a price. Many ethical concerns are voiced, related to the potential impact on animal welfare (Redmond, 2019). When rodents are bred with specific genes enhanced or knocked-out to understand, e.g. aggression or addiction, pain is intentionally inflected on these animals.

Embryos are seen as a replacement technique for live animal experiments (Okamoto *et al*., 2011; Van Soom *et al*., 2010). The EU Directive 2010/63/EU has defined an animal as "*any live non-*

human vertebrate animal or live cephalopod, from the time of fertilisation or from the time when development beyond the stage of primitive streak begins, until such time as any of the criteria for killing or humane endpoints set out in Annex II are reached" (European Union, 2010, Article 2(1)(d)). The primitive streak is a key developmental stage in which the embryo begins to form the basic structures that will eventually develop into the nervous system. In general, this stage occurs around 14 days after fertilization in mammals, and 24 hours after fertilization in birds (Bellairs, 1986; Sheng *et al.*, 2021; Stower & Bertocchini, 2017). Therefore, the use of non-human animal embryos in experimental research is allowed up to the primitive streak stage. These earliest life-stages are not protected, making the experiments not fall under the same framework of rules that live animal experiments do. If embryos were to be used after the primitive streak, it is considered to be the use of live animals and will be subjected to the same regulations as other animal research (European Union, 2010). Fish, in particular zebrafish, are one of the most common animals used for embryo studies. Zebrafish embryos are transparent, making it easier for researchers to observe their development and study the effects of various treatments (Karlsson *et al*., 2001). Not only are there less rules for fish in general, they also produce a high quantity of eggs per week, making it much more accessible and less expensive than other animal embryos (Redmond, 2019).

Embryo research is done in both humans as well as other animals. In animals, it is mostly used for studying regeneration in organs and other physical structures, as well as cloning (Gurley & Alvarado, 2008; Walters, 2004). Furthermore, stem cells are widely used in animal toxicology testing (Kim *et al*., 2019). Embryo research in humans is also increasing its potential, and is mostly aimed at creating therapies for treating diseases such as cancer, or to regenerate a damaged organ or tissue (Bhatia, 2001; Hunt, 2011; Trounson, 2009). Even though animal embryo research is advancing and creating more opportunities for alternative methods to animal testing, they cannot replace live animal experiments. There are furthermore many concerns regarding this ethically sensitive topic, specifically when it comes to human embryos in research. Animal embryos are included in the EU directive 2010/63, and a clear threshold has been installed for when it is considered a clump of cells and when it is considered a living organism. Still, the rules are much less stringent compared to human embryo research. Due to the fact that a human embryo can develop into a human being, the embryo itself has a moral standing (Assen *et al*., 2021). Furthermore, human embryos have to be created with use of gametes, for which a female and male participant are required. Therefore, strict rules are in place so that only when consent is given by both parties, can a human embryo be created and used in research (Lo *et al*., 2004). In the EU, countries such as Germany, Italy and Austria, heavily restrict the use of human embryos for scientific research (Matthews & Moralí, 2020), while other countries such as the United Kingdom and Belgium have more liberal policies regarding human embryos (Walters, 2004).

When it comes to non-human animals, there are no rules regarding consent in place. Of course, animals are not able to provide consent. Does that make it morally justifiable to then use animal embryos in research? If a human embryo has moral value because it can develop into a human, does the same not apply for other animal embryos which can also develop into living organisms? It is remarkable that even when it comes to clumps of cells from different species, there are different moral values and rights depending on which species the cells come from (Rosen, 2016). Not to say that scientist should abstain from animal embryo research, as both forms of research are very promising in providing new treatments and therapies for perhaps otherwise incurable diseases. However, animal embryos should receive the same respect, protection and safety in regards to harvesting and experiments as human embryos do (Steinbock, 2000).

Number of animals used in science

The EU Directive 2010/63 has been a very important step in a direction with less animal experiments. It has not only focused on better handle and care of animals in experiments, but also states the importance of finding alternatives and replacements for many animal experiments. Nevertheless, despite all new regulations, ethical considerations, and developments of non-animal studies, animals continue to be widely used in biomedical research. In seems that the total number of animals used in science has not decreased since the EU Directive. It is very difficult to estimate the total number of animals used in scientific research worldwide. Due to more legislation over the past decades, countries are obliged to report the amount and species of animals used in science in the EU (European Union, 2010). A study by Taylor and colleagues (2008) estimated the total number of animals used in

experiments worldwide around 58.3 million in 2005, before the EU Directive 2010/63. This number was derived by analysing the available reports, and using a prediction model for countries without national reports. However, they also included an extrapolation of a total of 115.3 million animals. This number accounted for all the animals used; the ones killed for tissues, animals used to maintain genetically modified species or strains, or those animals killed as surplus. In 2015, new statistics were published on the total number of animals used in science. Reports were obtained from a total of 37 countries, from which 30 were from Europe. For many countries, publications and models were used to determine the number of animals used. This resulted in 79.9 million animals used in scientific procedures across 179 countries (Taylor & Alvarez, 2019). Important to note is that this number does not include the number of animal experiments performed in Africa or South America, and only included reports from three countries from Asia. Furthermore, China is one of the biggest when it comes to science and animal experimentation, but does not provide any publicly accessible statistics (Taylor & Alvarez, 2019).

What is not included in many reports from countries regarding their animal experiments are all the animals/organisms used for the provision of tissue, transgenesis and embryo research, to maintain genetically modified colonies, or those bred for scientific purposes but not used. When the previous mentioned estimate was extrapolated to include these animals too, the total amount is increased to 192.1 million animals in 2015 (Taylor & Alvarez, 2019). This shows how important it is to report all animals for scientific research. Even though these animals might not be used for the actual experiments, they are still perceptible to experiencing stress under laboratory circumstances. Furthermore, whether they are used or not, they will eventually be killed.

The two reports from 2005 and 2015 have shown that there is a significant, and perhaps alarming, increase in the use of laboratory animals worldwide (Taylor *et al*., 2008; Taylor & Alvarez, 2019). For the EU, however, most countries are actually showing a decrease in the number of animals being used in experiments. That is a very positive trend, indicating that the EU Directive proves to be helpful in decreasing the amount of experiments. Nevertheless, there are some countries such as Spain, the United Kingdom, and Germany were the total number of animals used in experiments have increased (Taylor & Alvarez, 2019). This increase can be explained by the fact that there are indeed more animals being used, but it could also be explained by the new guidelines of the EU. Since the EU directive in 2010, it is mandatory to include genetically modified animals and animals used to maintain certain colonies (European Union, 2010). Including these animals does increase the total number of animals used in experiments per country. Therefore, it can be concluded that the EU Directive has a positive effect on decreasing animal experiments in Europe. Even though there have only been statistics calculated once before and once after the directive, it can provide an indication for the future. The estimate in 2015 was only two years after the directive actually became mandatory for all EU countries. If a new estimate were to be calculated, it would be expected to decrease even more. This would not only be due to the regulations being executed on every level, but also due to the fact that technology has increased tremendously after 2015, meaning that there are better alternatives on the market for animal experiments. Nevertheless, it is important to mention that the COVID pandemic probably has increased the total number again, as many animals were used to test the vaccines (Kalnin *et al*., 2021; Kostoff *et al*., 2020; Lurie *et al*., 2020).

Human bias

Humans have a very interesting way of assessing the value of different species. Whether we like it or not, most humans place species on a different place on the ladder based on their physiological features and their relationship to humans. This is quite similar to the *Scala Naturae* created by Aristotle (Fortenbaugh, 1971), or the speciesism concept conducted by Singer (Singer, 1996). For many centuries, humans were seen as the pinnacle of creation (Campbell & Hodos, 1991). When Charles Darwin introduced the evolution theory, this ideology became threatened. Darwin proposed that all species were created by evolution. He furthermore emphasized that evolution has no direction and is not biased, but is a random process which happens over time (Darwin, 1659). This means that humans were not specifically created to become a highly intelligent and cognitive species. Through evolutionary pressures, humans have developed certain skills that were necessary in order to survive. This is all based on the chance of mutations (Darwin, 1659). The same holds for all the other organisms on the planet. Every animal is adapted to its circumstances in the best way possible in order to increase the chances

of surviving (Alcock, 1972). In that way, every species is equal. It is true that humans possess qualities that are unlike any other that we see in the animal kingdom. Still, it is important to ask ourselves if we are even able to assess qualities in other species. We know that many animals communicate with each other through acoustic and biochemical communication (Ali & Morgan, 1990; Belanger & Corkum, 2009; Janik, 2009). Do humans not understand this type of communication because it is made by an animal who is "lower" on the phylogenetic tree of life? Or do humans lack the ability to understand this communication purely because we do not speak the language, and are not able to translate it to the human language, therefore lacking in our own abilities and intelligence? Just because something is not understood, does not mean other species lack intelligence or cognitive abilities. Even if other species are less intelligent than humans, does that give humans the right to control every organism on the planet?

This relates to the discussion of the moral status of animals. Some argue that animals have inherent moral values and should be treated with respect and compassion, while others argue that animals do not have the same moral status as humans (Rachels, 1990; Frey, 1988; Singer, 1996). The way humans handle and view animals is also predominantly based on the 'function' that these animals have in the lives of humans. For instance, dogs and cats are a regular meal for many Asian countries, especially China. When looking at the statistics of the countries who still use dogs for many experimental procedures, China is the number one of users of dogs, with the total number being estimated at 65,546 dogs per year (Taylor & Alvarez, 2019). For many countries in the EU, dogs are seen as companion animals, with whom humans can have emotional and strong connections. Dog experiments are still legal in the EU, however, the number of dogs used is much lower compared to for instance China. This is also evident with rodents, as most countries view these species, in particular rats and mice, as pests. The regulations regarding research on these species are much less stringent then the regulations for dogs or great apes, and they are still the most frequently used species in laboratories (Baumans, 2004; Baumans, 2016). Cultural, social, and moral values play an important part in human's opinion towards different species, and in what way there is a willingness to protect them.

Discussion

During the last 100 years, there have been many changes made regarding animal experiments. Not only have the experiments in itself changed, the way humans handle and look at animals have also changed. The paradox is that animal research itself created more knowledge that helped expand the regulations regarding experiments. When looking at the most important events during the last years, it is evident that between 1960 and 1980 many researchers, philosophers, and committees had a vital role in changing the perception and regulations. Ruth Harrison, Roger Brambell, and Peter Singer were all part of this movement (Broom, 2011; Franco, 2013). The 60s and 70s are signified by the many revolutions, which created the "*pivot of change*" (Elder, 2018). Social norms were changed, people lived more freely and equal rights were demanded (Taylor & Rupp, 1987). These revolutions caused more people to speak out, which also caused the discussion about animal welfare and animal rights as a whole to become more appealing. This is also evident in the evolution of the 3R's concept by Russel and Burch (Russell, 1995). When it was first published in 1959, there was a lot of critique on the whole concept and it did not become generally accepted. However, after a decade with significant changes in many fields, the concept regained attention at the beginning of the 70s. Now, some fifty years later, the 3R concept is still prominent in many laws and legislations concerning animal experiments.

As research on the cognitive abilities of many animals increased drastically in the first few decades of the 21st century, it caused the need for better legislation being executed on international levels. The Directive 2010/63/EU created even better standards for animal testing, and levelled the playing field more between countries. Animals with higher cognition or abilities to feel more pain, and species that have cultural or social values to humans have been given a sort of special status in international legislations (European Union, 2010). The laws are much stricter for these types of species, and the use of them can be seen as a 'last resort'. It does not mean that species like cats, dogs, or primates, are not used anymore or only very rarely (Patterson *et al.*, 2020). However, it ensures that other alternatives should first be explored, and the positive effects for humans should be weighed against the negative consequences for the animals. For animals with less cognition, or a lower phylogenetic order, there are also better guidelines created (Braithwaite & Huntingford, 2004; van Veen, 2021). Researchers should adhere these rules always to ensure the welfare of such species is

being respected. With the ethical commissions in place and the supervision of many governments, the welfare of animals in experiments have increased a lot.

The most important shift that has become evident in the past century but especially in the last two decades, is that there is much more attention and commotion surrounding animal welfare, animals in captivity or laboratories. This is not caused by only animal welfare activists or anti-vivisectionists, but researchers themselves are also becoming much more aware about handling the animals in experiments and trying to ensure their welfare. An important factor of today's world, is that the voice of society holds much more power. This can be seen in for instance the EU Citizens' Initiative (ECI). Through these initiatives, European citizenships can gather signatures for important topics that they want to bring in front of the European Commission. Once they have reached 1 million signatures, the European Commission is obligated to meet and discuss the topic. The "Save Cruelty Free Cosmetics – Commit to a Europe Without Animal Testing" European citizens' initiative was launched in 2021, has had over 1,2 million signatures from 22 different countries in Europe (Fentem, 2023). Never has an ECI reached that many signatures and has been so widely supported by so many different countries across Europe. Not only does this ECI focus on the ban of cosmetic testing on animals, it wants the European Commission to create legislation to ban all animal testing in the EU before the end of the current legislative term (Rivetti & Campos, 2023).

However, it is evident that as of right now, humans still 'need' animal experiments for science. If any situation made that incredibly clear, it was the COVID-19 crisis of the past years. Without animal experiments, the vaccines would have never been able to be developed this quick (Kalnin *et al*., 2021; Kostoff *et al*., 2020; Lurie *et al*., 2020). Even though there are already a lot of good alternative methods available and continue to be made, it is hard to know if humans could ever transition into a world without laboratory animals. Science as we know it is built on the backs on all the animals used in experiments. Therefore, the question remains if it is manageable to ban all animal experiments in the near future, as the ECI demands. On a practical level, it is impossible to house all animals used in experiments in other facilities where they can live out the rest of their lives. Killing all the animals in laboratories is not a solution either. Furthermore, science is still very dependent on animal experiments. The medicine we use daily are created through the help of animal experiments. With the increased risk of more pandemics in the near future, animals will be as important as ever to test vaccines on. Banning all animal experiments could stagnate science and the human health system. Lastly, even if the EU could ban all animal experiments, it does not mean that animal experiments over the entire world will be abolished. There are many countries, like China, were the conditions for animals in laboratories are much worse than in Europe (Taylor & Alvarez, 2019). It is impossible to know the entire laboratorial circumstances for these animals. The problem is then not resolved, but just transferred to other continents.

In the near future, it can be somewhat expected to see a decrease in animal experiments in the EU. This trend was already slightly visible in the statistical reports from Alvarez and colleagues (2019) in which it was evident what the effect of stricter rules had on animal experiments. Furthermore, the ever-expending field of technology continues to create alternatives and replacement methods for animal experiments. There is a new field on the rise in which bioacoustics and artificial intelligence could be used to communicate with animals (Bakker, 2022a; 2022b; 2022c). This would enhance the ethical discussions around animal experiments even more, as animals might finally be able to have a say in the matter. Adding to that, the concept and understanding of animal welfare is continuously being expanded too. A recent study by Arndt and colleagues (2022) have addressed a new dynamic concept of animal welfare (DAWCon), in which different factors are considered. It states that "*An individual is likely in a positive welfare state when it is mentally and physically capable and possesses the ability and opportunity to react adequately to sporadic or lasting appetitive and adverse internal and external stimuli, events, and conditions. Adequate reactions are elements of an animal's normal behaviour. They allow the animal to cope with and adapt to the demand of the (prevailing) environmental circumstances, enabling it to reach a state that it perceives as positive, i.e., that evokes positive emotion" (*Arndt *et al*., 2022, p.3). This new concept shows the evolution of animal welfare. Not only does it focus on the negative emotions, but it finds a continuum between positive and negative welfare. It addresses that welfare is different and dynamic, depending on the environmental circumstances and the capacity of adaptation of the animal (Arndt *et al*., 2022). When concepts like this are integrated in international legislations regarding experiments, the welfare of these animals will increase as well.

All these new concepts, initiatives, and technologies will help reduce the number of animals used in laboratories, and promises a better future for these animals. Nevertheless, it is not expected that animal experiments will be banned anytime soon in the near future. There are many other factors at play when it comes to the complete banning of animal experiments. Diseases, pandemics, social and cultural factors, as well as (inter)national laws all influence the future of animal experiments. Humans need to find a way to regulate all these different factors while also trying to decrease animal experiments.

Author's note

This thesis was written mostly based on the history, literature, and other facts concerning animal experiments. However, I feel it would not be complete if I did not include my own opinion. Let me start by saying everyone has the right to have their own opinion. Mine is without a doubt biased, by my upbringing, morals and values, and own experiences. If I was asked five years ago what my opinion was regarding animal experiments is, I would have told you that I am for completely abolishing all animal experiments. I would furthermore tell you that I think animal experiments in general are never morally justified. However, I've grown and learned as a person as well as a researcher. I now understand that there is much more to this topic than I might have cared to admit five years ago. I understand the value of animal experiments to science, but also to our everyday life. I would be naïve and ignorant to think that products that I use, the information that I gained from my studies, medicines I might use, or many basic facts about life would not have been possible without the use of animal experiments. I understand and appreciate their value, now more than ever. The alternative methods and replacement technologies are growing and expanding, and in my opinion, that should be our main focus. Nevertheless, I agree that the alternative methods are not yet on a level which can make sure all animal experiments can be banned. It is too short sighted to end all animal experiments today, this month, or even this year. Not only is that not possible because every continent or country in the world has its own moral believes and systems, it is also not manageable for all the animals currently still in laboratories. There would be no place to house them to live out the rest of their lives comfortably. That is why we should focus on scaling down this sector and the animals year by year. Only in that way, it is possible to sustainable reduce the animals, continue with the research, and work on better alternatives.

Nevertheless, I also feel a strong aversion towards the general idea of animal experiments. Something that has been evident since the beginning of human life is how much better we feel about our species in regards to other species. Most people forget that humans are animals themselves. I do not argue the fact that humans have developed into a creature that is highly intelligent and capable of many great things. Even though we drive high-tech cars, live in fancy houses and eat our food with cutlery, it does not mean that we too have very strong natural instincts connecting us to nature. What is not only clearly evident in the debate surrounding animal ethics, but in many problems the world nowadays face, is that humans have forgotten than we need nature more than nature needs us. Every single organism or species has a role to play in the ecosystem of the earth. Every species is important. Therefore, I do not think we are better than any other species. However, many people feel this way, whether it is intentional or not. Humans feel entitled to play God over all the other species. We decide when an animal is *good* or *bad*. We give species a moral value, a place on the imaginary ladder. We decide when the suffering an animal will experience during experiments outweigh the benefit humans can get from it. In my opinion, that makes us the most self-centred and egotistic species on the planet.

This is, of course, still my opinion, and it might come across as too harsh. However, I also came to some conclusions while writing this thesis that I feel are interesting to address. We have a different vocabulary when it comes to other animals. In scientific papers, an animal is never addressed at *he* or *she*, but as *it*. This creates the mindset that an animal is an object, not a being. Furthermore, in many ethical reviews, reports or legislation, the goal is to always treating the animals as *humanely* as possible. However, this word in itself signifies the problem. First of all, it is not possible to treat another animal, which is not human, humanely. This word can only be related to humans, as it is based on the concept of humans as a species. Second of all, humans would never treat another human in the same way that we treat animals. Nobody would ever allow their friend/partner/family member or any other human being to be put in a small cage. Even though the friend would have space to run around from time to time, and gets food and water occasionally or *ad libitum*, this would still not be considered a humanely treatment. Still, this is seen humanely for mice, rats, and many other small rodents in laboratories. There

are, however, no other words to describe the intention of the word *humanely*. That is one of the biggest paradoxes of our language.

Furthermore, if the world *humanely* would be adjusted to fit every species, it would for instance be possible to say "we treat a rat as '*rately'* as possible, or a primate as '*primately'* as possible". These words do not exist of course, however, they do provide a specific meaning or context. Generally speaking, it gives the impression that we should treat an animal, in this case a rat, as a rat deserves to be treated. For humans, rats have a particular reputation. They are perceived as pests, with a low degree of intelligence and cognition. For monkeys, it works the same way. Even though primates are generally seen as very intelligent animals with higher forms of intelligence and cognition, humans still perceive them as 'lower' animals. There is a bias, whether we want to admit it or not, when it comes to how humans think about and treat other animals. We treat other animals based on our own opinion and view of the animal, not with the actual value of the animal.

In my opinion, every species is considered equal in terms of rights to live and moral value. I do not find it morally justified to use and abuse other animals just for human profit. I do not think the world is balanced in that way, and I do not think it is healthy for the wellbeing of the planet. Do I think it is possible to live in such a world? Unfortunately, no. The world is way too complex, and we rely too much of animals for our health system and medicine to change this mindset. However, I very strongly believe that we can do the best we can to enhance animal welfare, and increase replacement methods so that we can move away from the animal experiments altogether.

Conclusion

Animal experiments have gone through many changes throughout the last century. The first few decades of the 20th century are highlighted by many breakthroughs in science mainly due to the use of animal experiments. All this new knowledge contributed to science as we know it today, and were vital in enhancing health care and medicines. The second half of the 20th century is highlighted by a lot of change regarding vivisection and animal rights. Not only did the concept of animal welfare became more enhanced and tangible, due to the efforts of behavioural researchers, more knowledge became available regarding the inner world of animals. On national as well as international level, new legislation became implemented to have stricter regulations in laboratories and to safeguard the welfare of animals. The EU directive 2010/63 was an important turning point. Stricter rules about many different species became implemented, levelling the playing field across countries. Furthermore, alternative methods are one the rise, creating more opportunities to have replacements for animals in experiments. The past 100 years have shown a significant change in not only the methodology, but also the mindset of people.

Nevertheless, the past years have also shown that animal experiments are still incredibly important when it comes to general science as well as health and medicines. This means that there is still room for improvement for species in laboratories. For as long as animals are still necessary, humans have to do all they can to enhance the living conditions of laboratory animals, and always keep their welfare as the number one priority. The ultimate goal is to abolish all animal experiments. However, this cannot be accomplished short term. Besides international legislation not being at the same level, it is also not possible to house all the animals currently in laboratories somewhere else. For the long-term, there should be a systematic downscale of animals in laboratories per year. Together with the increasing technology and replacement methods, the abolishing of animals in experiments can hopefully become reality in the next 30 to 40 years. Right now, it is important to still look critically at the way humans perceive and treat animals. At the end of the day, every animal deserves to be treated with respect.

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