Storyboard for developing Three Rs education resources

Replacement, Reduction, Refinement of animal use in science

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Disclaimer

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Introduction and Rationale

This resource is for teachers in secondary school and higher education. It aims to support development of curriculum activities relating to the replacement, reduction and refinement of the use of animals in science[1], and to explore the role of the European Union (EU) and the European Commission (EC) in working towards replacement.

A specific focus in this storyboard is the replacement of animal use in science, which is an important policy goal of the Europe Union as stated in EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

This resource will help in the organisation of debates with students on the history, the ethics or moral principles, the wider context of our relationships with animals, and looks ahead to new scientific innovations that will remove the need for animals in science.

This storyboard is one of the practical education resources. It aims to help educators develop wider debates with students, and discussion points are provided throughout the resource. It explains the rigorous requirements that have been established regarding welfare of experimental animals.

You are encouraged to develop debates with students on the ethical balances that occur in our relationships with animals. For example, the EU developments could be argued to show how the goal of replacement for scientific research purposes is aiming to maintain an ethical balance, and that it aims to contribute to the development of 'good science' in Europe[2] and beyond.

The storyboard is linked to a set of Powerpoint slides and a wider set of resources for teachers, such as secondary school and higher education learning scenarios, an online module (MOOC) for teachers and infographics for teachers to use.

Thanks to the support of a European Parliament Pilot Project to promote the Three Rs uptake in science, we are able to offer resources and a strategy to improve Three Rs teaching at secondary school, university and continuing professional development level.

> **The Three Rs**
> **Replacement** is defined as methods, strategies or approaches, which do not involve the use of live animals, for example in vitro systems using tissues or cells. **Reduction** refers to any approach that results in fewer animals being used to achieve the same objective, including maximising the information obtained per animal; reducing the number of animals used in a procedure; or limiting the subsequent use of additional animals. **Refinement** covers the modification of any procedures or housing and care practices to minimise the pain, suffering and distress experienced by the animal and to enhance its wellbeing. Refinement can also be achieved by moving from species that are considered more sentient to those less sentient.

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[1] Building on the concept of **Replace, Reduce, and Refine (the Three Rs)** principles in animal testing developed by Russell and Burch in '*The Principles of Humane Experimental Technique'* (1959)

[2] For example EU science and innovation https://www.youtube.com/watch?v=rtaJSpq-YmY Show the career opportunities and the range of science for the future https://www.youtube.com/watch?v=nKqJGoxkAUc

Background

Historically humans were regarded as being superior to animals[3] (we are part of a group of beings called 'primates'[4]), and therefore the use of animals for food, work and experimentation was acceptable. The earliest records of animal testing are from ancient Greece in the 4th and 3rd centuries BC, with Aristotle and Erasistratus among the first to perform experiments on animals. However, major issues arose in the early 20th century where new medicines or cosmetics were released onto the market without any prior knowledge of whether they were dangerous to humans. In effect the human consumers became the testers, with catastrophic consequences for some of them. Policy makers needed to reduce such risks and introduced the requirement that new products must be tested and shown to be 'safe' for humans before the products were allowed into the marketplace.

At that time using animals for scientific purposes was the obvious solution, and rats and mice in particular have been used for research and mandatory safety testing, along with other (nearer to human) animals such as non-human primates. The 'animal testing' paradigm has therefore been the one where the research and the business sectors have invested significant resources over the years. This paradigm argued that the 'alternative' to testing on humans was to test on animals.

Two outcomes have led to an increasing concern in society towards animal testing. One is that animal testing is not always successful and these models may yield results that cannot always be translated to humans. Consequently, around 90% of drugs fail in clinical trials, particularly in the area of cancer and Alzheimer's disease. Second, is a growing sense in society about animal rights. In 1822, the first animal protection law was enacted by the British parliament, followed by the Cruelty to Animals Act (1876), the first law specifically aimed at regulating animal testing. Nowadays we know more about animals, and research into their behaviour informs us more about their 'sentience', where animals feel emotion, pain, anxiety, show intelligence and the use of tools, and there are strong opinions that animals have 'rights' as we do as humans.

There is a third element to the societal understanding of animals, and that is the role of electronic communication channels, and the emergence of pro-animal organisations, that sensitise us much more to the actual testing processes, and to an awareness of adverse impacts on animals. Consequently, there has been the need for strong political attention, with the European Parliament Animal Welfare Intergroup[5] "*Founded in 1983, the Intergroup on the Welfare and Conservation of Animals was one of the first of the European Parliament's Intergroups to be established. For three decades it has provided a cross-party platform for [Members of the European Parliament] MEPs to discuss and exchange views on animal welfare issues*".

All of this has prompted significant efforts (such as funding through research programmes) to develop an 'alternatives to animal testing' paradigm, and new scientific innovations are

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[3] https://www.bbc.co.uk/religion/religions/christianity/christianethics/animals_1.shtml#h5
[4] https://australianmuseum.net.au/learn/science/human-evolution/humans-are-primates/
[5] https://www.animalwelfareintergroup.eu/

helping to move towards a paradigm change. This teaching resource elaborates some of these innovative approaches.

Replacement of animal testing is not something that is feasible overnight. There is a balance between reducing animal testing and not increasing the risks to humans.

So, we cannot realistically stop all animal testing immediately even if 'emotionally' we wish to do that. The EU policy response has therefore been to take (and enhance) an approach which follows a principle known as the Three Rs – replacement, reduction and refinement. The EU wants to **replace** animal use in science and will support research and innovation that develops new **alternatives** to the use of animals. This overriding principle has been accepted by all the Member States of the EU in the Directive agreed in September 2010[6].

What the Directive does, however, is to elaborate the principle to understand that as we move towards stopping all animal use for scientific reasons we must also ensure that any remaining animal use is **reduced** (there must, for example, be a clear scientific justification for the testing), and is **refined** (for example, those involved in the testing must show that any animal suffering is minimised, and that the animals are housed in as 'natural' an environment as possible).

[6] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32010L0063

How to use the Storyboard

The structure of the Storyboard

The storyboard is structured into themes and sub-themes, where the sub-themes are used to build slides in the slide set. Each sub-theme has a title and some detailed notes that are intended as material for teachers to develop their own knowledge, and to use in developing educational activities such as lesson plans.

There is no single journey through this **storyboard**, and it can be adapted to suit the needs of a specific curriculum or an educational level. It is the primary reference source that has been used to develop the accompanying **slide set**, and it aims to provide a 'ready-made' lesson material that may be modified to be introductory or more detailed.

The slides are left largely content-free, allowing material to be added that is directly relevant to the context being used.

For example, an overview of the material in secondary school European Studies context could use key points and discussion points to encourage debate. In a biology class one slide could be used several times to 'pace' a more detailed set of material.

There are also learning scenarios provided for use in secondary schools (training on how to use them is provided through ae Three Rs MOOC):

1. Animal welfare: animals in society, animals in science

➢ How society uses and misuses animals

➢ To use or not to use live animals in science

2. Sustainable science: the Three Rs, human-based science
➢ Sustainable science - The Three Rs

➢ Human-based science – Where humans can do it on their own

3. Critical thinking: debate acknowledging facts, emotion and science literacy

➢ Critical thinking: Emotions versus facts

➢ Animal experimentation in scientific literacy

These learning scenarios and six university-level learning scenarios can also be found here.

Secondary schools

Universities

Theme 1: Introduction and the scientific and social context

1. The use of animals - social and scientific context

The use of animals in our society - Slide 3

Humans have a long history of relationships with animals. We have long used animals for food, and we have kept them as domesticated beings. They have been used for centuries in activities related to hobbies and entertainment, such as being displayed in aquariums or zoos, in circuses where they often receive training. Hunting can also fall under both categories of providing for food and hobbies.

- Animals are **food** for humans, and the conditions under which they are kept, and the ways in which they are killed, can be controversial. There are also the issues about environmental impact of animals bred as food, such as in the contribution to greenhouse gases through our consumption of meat[7];

- Animals are **pets** (sometimes termed 'companion animals') for humans, but mostly we are not required to be 'trained' to have pets, and the existence of animal charities is a response to the suffering that we can inflict on animals;

- We use animals for **entertainment** (circuses for example, where animals are transported from one location to another), in **zoos** (where animals may not be housed in a 'natural' environment), for racing (horses, pigeons), and even for activities such as bull-fighting;

- We use animals as **labour**, for example working on farms, as military or police animals (detecting drugs or explosives etc.);

- We use animals for **assistance** (such as for blind people), or **emotional support** (for example pets being introduced into hospitals and care homes to provide therapeutic support).

Animals, including fish are used for nutrition; they are raised on land, factory farms or ocean-based aquafarms. Animal protection activists and organisations advocate for stricter regulations on quality, hygienic controls and animal welfare standards.

Domestic animals have been used as pets serving multiple purposes: assistance in case of health issues (guide dogs for visually impaired people, service animals for people with a disability, for example dogs helping diabetic patients), safety and companionship.

Cultures also differ in the way they treat animals as food:

- In India, cattle meat is not so popular due to the respect of cows in Hinduism. In turn, milk production is very high.

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[7] https://www.economist.com/graphic-detail/2019/11/15/how-much-would-giving-up-meat-help-the-environment
"*Going vegan for two-thirds of meals could cut food-related carbon emissions by 60%*"

- To eat pork is forbidden, but also shellfish is traditionally seen as unclean in Judaism (not kosher) and Islam (not halal except in some sects of Islam). Therefore, selling shellfish was historically an occupation for other minorities in the Middle East.
- There are exemptions in EU law to allow different methods of animal slaughter to take place for religious purposes.
- Although not all Buddhists avoid eating meat, many Buddhists are vegetarians for reasons of compassion for life.

Discussion points:

- *Compare statistics about the status of hunting in European countries but also in other parts of the world.*
- *Find relevant regulations on sustainable hunting initiatives (the European Commission is actively looking to foster dialogue on sustainable hunting of birds: see here) and suggest measures for better regulated and more responsible hunting activities.*
- *While pet ownership is widespread in many countries, there is a long tradition of looking after stray animals in countries like Turkey and Greece. Examine attitudes towards pet ownership in Europe and outside Europe. How do attitudes differ?*

The use of animals in science - Slide 4

The use of animals in **scientific research** emerged in the 3rd century BC, where a primary motive was to advance medical science without having to experiment on live humans. In the latter part of the 20th century we saw a major increase in animal use. Animals have even been used in aeronautic and space exploration.

Animal testing has been used for developing **new treatments and medicine** for both humans and animals. Testing involves carrying out safety, efficacy, and **risk-analysis with new chemicals** to be used in cosmetics, medicines, and vaccines. Animals are used for **education and training** purposes, such as training surgeons in developing their skills in invasive surgery. Vets practice on dead or alive animals. In some schools and university teaching students learn about the body structures of animals by dissecting dead ones.

We are seeking out **new 'cures'** for cancer, dementia and we will continue face new diseases. As we develop new medical solutions, we have to ask questions: does this drug work (this involves complex experiments on a range of 'subjects' who have the illness and do not have the illness) even if a new drug may cure an illness does it have bad side-effects? The experiments to evaluate such questions have often involved animals, but we are confronted by **changing societal views**, **scientific evidence** that the current animal testing paradigm needs to change, and **new technological innovation and scientific research** that help us explore opportunities to avoid using animals.

Discussion points:

- *Why does animal experimentation still take place? (for example, consider how new non-animal testing methods are approved for use or 'validated' – how and by whom validation or approval is undertaken is covered later in this resource).*
- *Who are the professionals involved in animal testing and what do they do? You can consider:*
  - *those whose businesses and research reputations are based on animal testing.*
  - *how research reputations are built and on what criteria.*
  - *where animal testing is addressed (or not) in education curricula.*
  - *the logistics and costs of changing a laboratory from an animal-based facility to a facility equipped to conduct non-animal approaches.*
  - *how governments are changing their regulatory approaches. and*
  - *explore the development of social activism and the growth of organisations promoting the replacement of animal testing.*

2. Why do we test on animals?

History of animal experimentation - Slide 5

We know that during early Greek civilization there were experiments taking place on live animals[8], and:

> *Galen (129 - c.217 AD), a Greek physician who practiced in Rome and was a giant in the history of medicine, conducted animal experiments to advance the understanding of anatomy, physiology, pathology, and pharmacology. Ibn Zuhr (Avenzoar), an Arab physician in twelfth century Moorish Spain, introduced animal testing as an experimental method for testing surgical procedures before applying them to human patients*.[9]

Roman physician Celsus, who lived between 25 BC and 50 AD, mentions in his writings the practice of vivisection which had taken place on convicted criminals. In Alexandria, Herophilus and Erasistratus (physicians, 3rd century BC) had engaged in the practice, disregarding the established taboos, in order to shed light on medical uncertainties and discover more about human anatomy. Celsus himself did not condone this, stating that "…to lay open the bodies of men is as cruel as it is needless."[10]

> *Claude Bernard, known as the father of physiology, stated that "experiments on animals are entirely conclusive for the toxicology and hygiene of man. The effects of these substances are the same on man as on animals, save for differences in degree". Bernard established animal experimentation as part of the standard scientific method.*[11]

The practice of testing cosmetics on animals started in the United States in the 1930s. It was triggered by events such as one involving "*Lash-Lure, an eyelash dye in which a number of women suffered injuries to their eyes, including one confirmed case of*

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[8] An example of a storyline for schools
http://www.softschools.com/timelines/history_of_animal_testing_timeline/266/ Try out a timeline maker:
https://www.ispringsolutions.com/blog/top-10-free-and-paid-interactive-timeline-makers
[9] For a full discussion see the articles at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3123518/ and
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4495509/
[10] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5117177/
[11] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3123518/

permanent blindness."[12] The response was the passing of the US Federal Food, Drug, and Cosmetic Act in 1938, which required that ingredients and finished cosmetic products (also drugs and medical devices) must be proven to be safe prior to marketing. The Act requires that manufacturers take the necessary steps to ensure the safety of their products. This did not exclude testing on live animals.[13]

Following this tests on animals were developed, such as the 'Draize Test', where a substance is dropped into the eyes of an animal and/or smeared onto its shaved skin. The LD 50 (Lethal Dose 50) test involves applying increasing amounts of a substance to a group of animals until half of them die.

Research has shown that neither of the above tests are scientifically proven to be good predictors of the reactions in human beings, and in addition there were strong views that animals in experimental conditions were under stress and therefore did not represent their 'normal state'. 'Normal' refers to physiological and behavioural conditions reflecting the usual conditions in which an animal would live, ensuring a good state of health. This good state of health is essential for control (reference) animals. Furthermore, in experiments the 'control data' on many animal species was from animals that were not in a 'normal state.'

Significant improvements have been made in animal housing and welfare, aiming to minimise stress. Nevertheless, in recent years, the practice of using animals for biomedical research has come under severe criticism by animal protection and animal rights groups. Companies like the Body Shop have paved the way for testing cosmetic products without using animals. Also, the book by ethicist Peter Singer, '*Animal Liberation: A New Ethics for Our Treatment of Animals*'[14], where he elaborated the topic 'speciesism', which implies that humans always come first, has had a significant influence on the way we view animals.

Laws have been passed in several countries to make animal testing more 'humane'. Debates on the ethics of animal testing have even been a concern of US President Theodore Roosevelt, who stated, "*Common sense without conscience may lead to crime, but conscience without common sense may lead to folly, which is the handmaiden of crime*."

In Europe, using animals for cosmetic testing is no longer allowed (see section 3.3).

> *Discussion point: What do you understand by 'ethics'? What 'ethical principles' would you regard as being important for scientific research?*

> *Discussion point: Read the book 'Dying to Be Beautiful: The Fight for Safe Cosmetics*'[15] *by Gwen Kay to know more about Hazel Fay Musser, a 10-year-old child, who played an important role in the approval of the US Federal Food, Drug, and Cosmetic Act in 1938.*

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[12] https://www.fda.gov/about-fda/fdas-evolving-regulatory-powers/part-ii-1938-food-drug-cosmetic-act
[13] https://www.fda.gov/cosmetics/resources-consumers-cosmetics/cosmetics-safety-qa-animal-testing#:~:text=Does%20FDA%20require%20animal%20testing,cosmetics%20to%20FDA%20premarket%20approval.
[14] https://en.wikipedia.org/wiki/Animal_Liberation_(book)
[15] https://www.slideshare.net/v2zq/yzd162

3. Replace, Reduce, Refine: Using animals for scientific purposes in the EU

The principle of the Three Rs - Slide 6

In the late 1950s concern about animals suffering while being used in scientific experiments led to two researchers (William Russell and Rex Burch) to publish a book, '*The Principles of Humane Experimental Technique*'[16] (1959) where they developed the concept of the Three Rs - Replacement, Reduction and Refinement. The Three Rs then became widely adopted as important **ethical principles** for scientific research. The Three Rs involve:

- Replacement. Replacing the use of live animals, e.g. by using computer data (*in silico*), human cells (*in vitro*) and using *ex-vivo* (meaning that the testing takes place outside an organism) techniques;
- Reduction. Obtaining the same amount of information through using fewer animals, or more information using the same number of animals; this can be reached by better statistics and improved literature searches;
- Refinement. Reducing the discomfort (pain and suffering), for example by providing good anaesthesia and analgesia, and improving the welfare of the animals, e.g. by providing social housing (only for species that are by nature social) and cage enrichment.

The principle of the Three Rs was firmly established in EU legislation through Directive 2010/63/EU with the ultimate goal to replace animal testing in science.

In short, the Three Rs contribute to better animal welfare and better science, thereby increasing the value of research.

EU statistics on the use of animals for scientific purposes - Slide 7

The most recent statistics about animals used in scientific research in the European Union were published in February 2020, and they relate to the period 2015-2017.[17] There are three distinctive areas where statistics are now reported:

1. Numbers of animals used for research, testing, routine production and educational (including training) purposes). These animals can be both conventional animals or those that are genetically altered (GA)[18].

2. Details of all uses (first and any subsequent reuse) of animals for research and testing. This serves to draw an overall picture of all 'uses' of animals for scientific purposes and takes into account the nature of the procedures, their legislative context, reuse of animals, the genetic status of the animals, and the severities experienced by the animal.

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[16] http://altweb.jhsph.edu/pubs/books/humane_exp/het-toc

[17] https://ec.europa.eu/environment/chemicals/lab_animals/reports_en.htm

[18] A term used to describe animals that have had their genes changed in some way in order to study a disease or the function of a gene. In science these animals are usually rats or mice.

3. Numbers and uses of animals for the creation and maintenance of genetically altered animal lines. The third section focuses on the provision of genetically altered animals needed for science in the EU. These animals have not been used in other scientific procedures, covered in sections one and two above.

Key statistics from the report are:

- Total numbers of animals used for the first time for research, testing, routine production and education purposes across the three years were: 9,590,379 (2015), 9,817,946 (2016), and 9,388,162 (2017).

- Total number of animals used for the creation and maintenance of genetically altered animal lines: 1,588,025 (2015), 1,193,692 (2016), and 1,276,587 (2017).

- The breakdown of species of the 9,388,162 animals used in 2017 is provided in the report in its Figure 1 and Table 3, with the summary explanation being: "In 2017, the main species used for the first time in research and testing were mice, fish, rats and birds, which together represented 92% of the total number of animals while species of particular public concern (dogs, cats and non-human primates) represented less than 0.3% of the total number of animals. No Great Apes are used for scientific purposes in the EU". The 2017 statistics are presented below from the highest to the lowest use.

Numbers of animals used for the first time by species	2017	% in 2017
Mice	5,707,471	60.79
Rats	1,146,299	12.21
Other Fish	719,932	7.67
Zebra fish	499,763	5.32
Domestic fowl	464,553	4.95
Rabbits	351,961	3.75
Guinea-Pigs	144,824	1.54
Other birds	99,410	1.06
Pigs	71,522	0.76
Cattle	30,643	0.33
Other mammals	26,335	0.28
Other rodents	25,172	0.27
Sheep	18,812	0.20
Dogs	13,688	0.15
Xenopus	13,539	0.14
Hamsters (Syrian)	12,700	0.14
Other amphibians	10,683	0.11
Cynomolgus monkey	7,227	0.08
Mongolian gerbil	5,239	0.06
Rana	3,485	0.04
Reptiles	2,937	0.03
Horses, donkeys and cross-breeds	2,414	0.03
Other carnivores	2,386	0.03
Ferrets	2,016	0.02
Cats	1,879	0.02
Goats	1,563	0.02
Cephalopods	514	0.01
Marmoset and tamarins	465	0.00
Rhesus monkey	353	0.00
Hamsters (Chinese)	187	0.00
Prosimians	98	0.00
Vervets (Chlorocebus spp.)	33	0.00
Baboons	25	0.00
Other species of Old World Monkeys (Cercopithecoidea)	23	0.00
Squirrel monkey	8	0.00
Other species of New World Monkeys (Ceboidea)	3	0.00
Total	**9,388,162**	100.0

Discussion points:
- *Can you find out how many animals were used in safety and risk assessment for food and feed?*
- *What types of scientific research would use animals? What is the difference between 'basic research' and 'translational and applied research'?*
- *How many animals were used for scientific purposes in your country[19]?*
- *Search your national media for coverage of the EU statistics. What interpretations and viewpoints are being reported? How can you objectively balance the viewpoints?*

[19] The European Animal Research Association summarises the statistics at https://www.eara.eu/animal-research-statistics?lang=nl

4. Our relationships with animals

European Citizens' Initiative (ECI) "Stop Vivisection" - Slide 8

We have become much more aware of the ethical, scientific, legal and economic reasons to minimise the use of animals in scientific research. However, we do use animals for many other purposes, such as to help overcome disabilities (such as assistance dogs) and for emotional support.

Public opinion has grown stronger about the need to stop using animals in scientific research, for example in the European Citizens Initiative of 2015, signed by 1.17 million citizens, and where the European Commission responded by emphasising the goals of the Directive[20]. A European Parliament intergroup[21] is formed when Members of the European Parliament come together to address a particular topic. The topic may be outside the normal agenda of the Parliament and the group is formed between political parties. The Intergroup for the Welfare and Conservation of Animals currently has 97 members[22], and this reflects the level of concern among both politicians and citizens on this subject. Statistics about animal testing are communicated extensively via the Web[23], sensitising people much more readily to the extent of animal testing. The open access resource ALTEX (Alternatives to Animal Experimentation)[24] promotes the publication of academic research related to animal alternatives. This is one example amidst the large amount of scientific literature (research) available on Three Rs approaches.

Regulating the use of animals emerged as governments aimed to reduce unnecessary suffering.

The Treaty on the Functioning of the European Union (TFEU) Article 13 states that:

> *In formulating and implementing the Union's agriculture, fisheries, transport, internal market, research and technological development and space policies, the Union and the Member States shall, since animals are **sentient beings**, pay full regard to the welfare requirements of animals, while respecting the legislative or administrative provisions and customs of the Member States relating in particular to religious rites, cultural traditions and regional heritage.*[25]

Being sentient means animals "…have the capacity to experience positive and negative feelings such as pleasure, joy, pain and distress that matter to the individual."[26] In other words they possess an awareness of both positive and negative feelings and emotions.

Discussion points:

- *What do you understand by the word 'vivisection'?*
- *What do you understand as 'sentience' in ourselves and in animals – how can we and they 'suffer'?*

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[20] https://europa.eu/rapid/press-release_IP-15-5094_en.htm

[21] https://www.europarl.europa.eu/news/en/faq/10/what-are-intergroups-and-how-are-they-formed

[22] https://www.animalwelfareintergroup.eu/

[23] For example https://speakingofresearch.com/facts/eu-statistics-animal-research/ and https://www.altex.org/index.php/altex/article/view/145

[24] https://www.altex.org/index.php/altex

[25] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A12012E%2FTXT

[26] https://science.rspca.org.uk/sciencegroup/sentience

- *What do you understand by animal rights?*
- *What aspects of animal use could be covered by 'religious rites, cultural traditions and regional heritage.'*

Theme 2: The European Union and Animals Used for Scientific Purposes

1. Why has the European Union acted to accelerate the development of alternatives to the use of animals?

EU law and animal use in science - Slide 10

There are a number of ways that the EU can influence what happens across Member States, ranging across Regulations, Directives, Decisions, Recommendations, and Opinions[27]. An EU Regulation is legislation that is binding and applies to all Member States equally. A Directive is legislation that sets out the policy goals, but Member States are then required to either implement the Directive in their own laws, or to develop new national legislation to achieve those goals. That process is called transposition:

> *For a directive to take effect at national level, EU countries must adopt a law to transpose it. This national measure must achieve the objectives set by the directive. National authorities must communicate these measures to the European Commission. Transposition must take place by the deadline set when the directive is adopted (generally within two years).*[28]

European society has become much more aware of the ethical, scientific, legal and economic reasons to minimise the use of animals in scientific research. As a result, EU Member States have been sensitive to changing societal views about the use of animals in research or safety testing.

> *Discussion point: Why should there be consideration of this issue at the European level? Consider such aspects as the 'Single Market' and the need to make sure that consumer goods can be sold in all Member States, so if one of them demands 'animal free' testing it is important that this applies to all, otherwise some products could not be purchased in some countries.*

Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes first introduced the Three Rs principle into EU legislation. From 1993 onwards the European Commission also wanted to stop the use of animals in testing cosmetics. In such tests, the chemicals used in cosmetics may be dropped into the eyes, or rubbed onto the shaved skin of animals such as rabbits. They may be forced to swallow the substances to be tested. The tests aim to identify whether they develop illnesses, whether they lead to cancer or birth defects, or to understand what level of chemical can lead to death. The animals may suffer eye problems (even blindness), physical problems, and other complications leading to death.

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[27] https://europa.eu/european-union/law_en
[28] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=LEGISSUM%3Al14527

Stopping the use of animals in testing cosmetics required that 'alternative' methods of testing would be available. As a result, the development of legislation was incremental: for example, first banning the testing of finished products, and leading in 2013 to a ban on any use of animals in the development and testing of cosmetics.[29] Under the Cosmetics Regulation (EC, 2009), if a finished cosmetic product or any of the ingredients contained in it has been tested on animals since 2013, it is banned from the EU market whether it was produced within the EU or elsewhere.

Between 2007 and 2011, to accelerate the development of alternative methods the European Commission provided 238 million euros of funding for projects which developed non-animal approaches for cosmetics. For teaching purposes these developments are visualised in the form of a rabbit.[30]

The use of animals in the testing of food has been the focus for the European Food Safety Authority (EFSA).[31] For example, it has provided:

> *guidance for carrying out feeding trials related to applications for novel foods and for food and feed derived from genetically modified plants. The guidance outlines experimental designs aimed at ensuring relevant results while minimising the number of animals to be used*.

And:

> *In the field of risk assessment of food additives and pesticides, EFSA has adopted a 'tiered approach'[32] that guides applicants in designing their toxicological testing strategy. Decisions are based and justified on scientific evaluation of the results in stages. Such an approach can result in the need for fewer or more refined animal tests*.

> *Discussion point: do you check whether cosmetics you use are free of all animal testing? How can you check? You could also ask what is included under the term 'cosmetics' – the definition is provided in an above footnote, but it would be interesting to see how many students identify soap, toothpaste etc. as being included.*

2. Animal welfare

An EU value - Slide 11

Article 13 of the Treaty on the Functioning of the European Union acknowledges the rights of animals:

> In formulating and implementing the Union's agriculture, fisheries, transport, internal market, research and technological development and space policies, the Union and the Member States shall, since animals are sentient beings, pay full regard to the welfare requirements of animals, while respecting the legislative or

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[29] https://ec.europa.eu/growth/sectors/cosmetics/animal-testing_en
[30] https://ec.europa.eu/growth/sites/growth/files/rabbit-05.jpg
[31] https://www.efsa.europa.eu/en/topics/topic/alternatives-animal-testing
[32] A tiered approach uses data already available and simple biological methods first in a hierarchy of tiers. The aim is to maximise efficiency and minimise the use of animals in toxicological assessment.

administrative provisions and customs of the Member States relating in particular to religious rites, cultural traditions and regional heritage.

The EU has no powers to legislate on the basis of animal welfare. Animal welfare is the responsibility of Member States.

However, animal welfare must be taken into account when formulating all European Union policy. In the case of Directive 2010/63/EU it was to ensure harmonisation of the functioning of the internal market.

The EU Treaties including the Treaty on the Functioning of the European Union can be found here (The Directive preamble refers to Article 114).

3. Directive 2010/63/EU on the protection of animals used for scientific purposes

Subject matter and scope, definitions and requirements - Slides 12 & 13

Directive 2010/63/EU lays down rules for the protection of animals used for scientific purposes. Within the European Commission Directorate-General Environment leads the file on Directive 2010/63/EU, the related website should be consulted for updates on implementation, statistics, events and other activities. Some informative posters and flyers about the Directive can be found here.

Article 1 of Directive 2010/63/EU defines the subject matter and scope of the legislation. Within the scope of the legislation are live non-human vertebrate animals, independently feeding larval forms, foetuses of mammalian species in their last trimester of development and cephalopods (squid, octopus). Live animals may only be used for the purposes of research, regulatory testing, higher education and training.

Article 3 of Directive 2010/63/EU defines a procedure carried out on a live animal:

> 'procedure' means any use, invasive or non-invasive, of an animal for experimental or other scientific purposes, with known or unknown outcome, or educational purposes, which may cause the animal a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice.

The legislation applies to all EU Member States and is firmly based on the principle of the Three Rs, to replace, reduce and refine the use of animals used for scientific purposes. Member States had to 'transpose' (i.e. implement) the Directive into their national legislation by 10 November 2012.

Directive 2010/63/EU in 23 languages can be found here

> *Discussion point: How was the Directive implemented in your country? For example, what is the title of the legislation, which bodies are responsible for the legislation? Start your research on the European Commission website for Directive 2010/63/EU*

Discussion point: Was the Directive transposed into existing legislation or was new legislation created?

Discussion point: Imagine that you are a policy maker in your country. How would you ensure full implementation and full compliance of the legislation?

The Directive includes measures on:

- Endangered species (Article 7);

- Non-human primates (Article 8);

- Animals taken from the wild (Article 9);

- The Directive covers all animals in line with its Article 1. Annex I specifies those species that have to be purpose bred when used for scientific purposes. Animals purpose bred for use in scientific procedures (Article 10/Annex I) are Mouse (*Mus musculus*), Rat (*Rattus norvegicus*), Guinea pig (*Cavia porcellus*), Syrian (golden) Hamster (*Mesocricetus auratus*), Chinese hamster (*Cricetulus griseus*), Mongolian gerbil (*Meriones unguiculatus*), Rabbit (*Oryctolagus cuniculus*), Dog (*Canis familiaris*), Cat (*Felis catus*), All species of non-human primates, (*Xenopus* (*laevis*, *tropicalis*), Rana (*temporaria, pipiens*)), Zebra fish (*Danio rerio*);

- Stray and feral animals of domestic species (Article 11).

The Directive in a nutshell:

Directive 2010/63/EU

In the EU, **live vertebrate animals** and **cephalopods** can **only** be used for **specified scientific purposes:**
- once the **project** is **authorised**
- by a **competent authority**
- having **evaluated** that **harms are justified by the expected benefits** taking into account **ethical considerations**, <u>and</u>

- the work is carried out by **trained and competent staff**,
- according to the **rules and conditions** set by the Directive,
- in a **regularly inspected** and **authorised establishment**
- in compliance with **housing and care standards** and with
- mandatory **persons** and **structures** for **animal health & welfare**.

Why an EU directive? - Slide 14

The EU legislates across Member States through Regulations and Directives. A Regulation is legislation that applies to all Member States equally. A Directive is legislation that sets out the policy goals, but Member States are then required to either implement the Directive in their own laws, or to develop new national legislation. That process is called transposition.

A Directive covering animals used for scientific purposes had been in place for 25 years (86/609/EEC) and thanks to this Member States were already carrying out some of the requirements under the new legislation 2010/63/EU. For this reason a new Directive, replacing the old one (86/609/EEC) was considered more suitable and allowed a flexible implementation for Member States.

The Directive and the Three Rs - Slide 15

Directive 2010/63/EU introduced legislation that applies to all EU Member States regarding the protection of animals for scientific purposes. It has firmly established the principle of the Three Rs (replacement, reduction, refinement of animal use in science) within EU legislation.

The Directive states how the Three Rs must be applied (see Recital 10 and Articles 4 and 13). It is a legal requirement to apply the Three Rs in all aspects of animal use in science – breeding, housing and care, use in procedures and killing.

The introduction of this principle also embodies the concept of alternatives to animal use, which are test methods, techniques or tools as well as strategies, activities or approaches.

> **The Three Rs**
> **Replacement** is defined as methods, strategies or approaches, which do not involve the use of live animals, for example in vitro systems using tissues or cells. **Reduction** refers to any approach that results in fewer animals being used to achieve the same objective, including maximising the information obtained per animal; reducing the number of animals used in a procedure; or limiting the subsequent use of additional animals. **Refinement** covers the modification of any procedures or housing and care practices to minimise the pain, suffering and distress experienced by the animal and to enhance its wellbeing. Refinement can also be achieved by moving from species that are considered more sentient to those less sentient.

1. **Replacement:** Wherever it is possible, Member States must aim to replace the use of live animals with 'a scientifically satisfactory method or testing strategy.'

Article 13 and Article 4 of Directive 2010/63/EU emphasise that replacement must take place '*wherever possible*'. Replacement methods must be **scientifically satisfactory** and they must produce the information required. The scientific aim of the study must be clearly understood when considering possible replacements. For example, in a case where human welfare is the aim of research, then a replacement based on human cell lines would be more appropriate than one that uses animal cell lines.

The validation (or confirmation that a test is suitable for its purpose) of a replacement method should be carried out in the target species i.e. validated against a replacement model based on human cells/tissues/organs if the target is a human disease. It should not be sufficient to validate or approve against an existing animal model even if that model has long been considered as the 'gold standard' in drug discovery.

While efforts have been made to ensure the uptake of the Three Rs across teaching, learning, and scientific research, it has been uneven across Member States. Recently, the European Parliament has invested significant funding to develop teaching, learning, and research resources at the European level to accelerate the European-wide implementation of the Three Rs.

The final goal of Directive 2010/63/EU is:

"…full replacement of procedures on live animals for scientific and educational purposes as soon as it is scientifically possible to do so."

> *Discussion point: What does validation mean and why is it significant?*
>
> *Discussion point: Explore what the Directive states about 'validation' (Article 47, and Annex VII). Then look at the range of replacement methods that have been validated[33].*
>
> *Discussion point: How do I find out what is happening with the Three Rs in my own country? Look at the national centres that are promoting the Three Rs.[34] Contact them to ask about their work.*
>
> *Discussion point: Can you list some examples of 'replacement'?*

2. **Reduction:** The Directive acknowledges that some scientific experiments still need to use animals, it is essential that the number of animals used is reduced to a minimum whilst still obtaining the level of information required. For example, by analysing the scientific literature completely and objectively (for example through a method called 'systematic review'), unnecessary duplication of animal studies can be prevented, and new results and new insights are obtained without doing new animal studies.[35] An example from Syrcle (the SYstematic Review Center for Laboratory Animal Experimentation) at Radboud University Medical Centre is where undertaking an increased number of systematic reviews, the number of animal experiments carried out by the University of

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[33] https://ec.europa.eu/jrc/en/eurl/ecvam/alternative-methods-toxicity-testing/validated-test-methods
[34] https://ec.europa.eu/jrc/en/eurl/ecvam/knowledge-sharing-3rs/knowledge-networks/eu-3rs-centres
[35] www.syrcle.nl

Nijmegen reduced (between 1999-2005 and 2005-2014) by 30% (with no reduction in published output for the researchers), as against 15% for the Netherlands as a whole.

> *Discussion point: How do we know whether reduction is taking place, and at what rate? Note the progress reports[36] on the DG Environment site, and an ongoing review of progress by the JRC[37] EURL ECVAM Status Report 2019.[38]*

> *Discussion point: Can you list some examples of 'reduction'?*

3.   **Refinement:** Where animals are still being used, ensure refinement of breeding, accommodation and care, and of methods used in procedures, eliminating or reducing to the minimum any possible pain, suffering, distress or lasting harm to the animals, and aim to improve the welfare of the animals.

> *Discussion point: Can you list some examples of 'refinement'?*

> *Discussion point: How could we minimise pain, improve the housing and care of animals? Does social housing instead of individual housing always lead to improved welfare?*

> *Discussion point: Take one animal from the list in Annex I of the Directive and explore the housing and other animal husbandry conditions that are required referring to Annex III and what could be changed to refine the conditions.*

> *Discussion point: What species would not be included under live vertebrates and cephalopods, according to the Directive?*

> *Discussion point: How would we know that someone is 'trained and competent'?*

> *Discussion point: Where are animals used for education purposes (schools, higher education, training)? Connect with secondary schools and universities to find out.*

> *Discussion point: How would an establishment be 'authorised', and how could it be inspected?*

> *Discussion point: How do I find out what is happening with the Three Rs in my own country? Look at the [organisations]() that promote the Three Rs*

New legislation highlights 1/2 - Slide 16

Directive 86/609/EEC the protection of animals used for experimental and other scientific purposes **was replaced by Directive 2010/63/EU** on the protection of animals used for scientific purposes, widening the scope and strengthening the protection given to experimental animals. Directive 2010/63/EU includes foetuses of mammalian species in their last trimester of development and cephalopods, as well as live non-human vertebrate animals used for the purposes of basic research, higher education and training. It lays down **minimum standards for housing and care** (see Annex III of the Directive), regulates the use of animals through a systematic **project evaluation** (see also the slide

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[36] https://ec.europa.eu/environment/chemicals/lab_animals/3r/advance_en.htm

[37] https://ec.europa.eu/jrc/en/eurl/ecvam/knowledge-sharing-3rs/life-science-research

[38] https://ec.europa.eu/jrc/en/publication/eur-scientific-and-technical-research-reports/eurl-ecvam-status-report-development-validation-and-regulatory-acceptance-alternative-4

on 'application for project authorisation') requiring *inter alia* assessment of pain, suffering distress and lasting harm caused to the animals.

Inspections – Article 34

Article 34 requires Member States to '*ensure that the competent authorities carry out regular inspections of all breeders, suppliers and users, including their establishments, to verify compliance with the requirements of this Directive*.' The frequency of inspections should be undertaken on the basis of a risk analysis for each establishment, rather than on a fixed time period. The risk analysis should take account of: 'the number and species of animals housed; the record of the breeder, supplier or user in complying with the requirements of this Directive; the number and types of projects carried out by the user in question; and any information that might indicate non-compliance.'

> *Discussion point: What is the difference between a regular programme of inspections and a programme based on risk analysis?*

Member States must inspect at least one-third of the users each year, with the exception that '*breeders, suppliers and users of non-human primates shall be inspected at least once a year.'* There is a requirement that '*an appropriate proportion of the inspections shall be carried out without prior warning. Records of all inspections shall be kept for at least 5 years.'*

Article 35 relates to '*controls of Member State inspections*', noting that while the Member States are responsible for carrying out inspections, the Commission also needs to be confident that the inspection programmes are appropriate and meeting the obligations laid out by the Directive.

The Commission may have '*due reason for concern, taking into account,* inter alia*, the proportion of inspections carried out without prior warning, undertake controls of the infrastructure and operation of national inspections in Member States*.'

In such circumstances the Member State must assist the experts who are appointed by the Commission to carry out this action, and they will inform the Commission of their recommendations, and the Commission will inform the '*competent authority of the Member State … [which] shall take measures to take account of the results of the control.*'

> *Discussion point: Does this process ensure that there is independent oversight of the Member State inspections? The Commission will control Member State inspection programmes and infrastructure, not animal establishments directly.*

Non-technical project summary – Article 43

Article 43 requires a **non-technical project summary** to be prepared and submitted with the project proposal. It should describe the project in a way the lay person can understand, including information on the objectives of the project and how the Three Rs have been applied, and specify whether a retrospective assessment is needed. It should be made publicly available. **Decision 2020/569 on Reporting** provides a mandatory template for

non-technical project summaries. In 2021 the Commission will provide a searchable, public database where all the NTS can be found.

Application for project authorisation - Slides 18 & 19

Directive 2010/63/EU requires that all projects involving animals must be formally authorised (Chapter 4, Article 36), which entails that proposers must submit an **application** to be **authorised** by a '**competent authority**'[39] which is '*an authority or authorities or bodies designated by a Member State to carry out the obligations arising from this Directive*.'

It is the responsibility of each Member State to ensure that projects do not start without authorisation being granted, and that the projects are actually carried out by strictly following the authorisation. There is a *caveat* (Article 42) where:

> "*Member States may decide to introduce a **simplified administrative procedure** for projects containing procedures classified as 'non-recovery', 'mild' or 'moderate' and not using non-human primates, that are necessary to satisfy regulatory requirements, or which use animals for production or diagnostic purposes with established methods*."

Article 37 defines the structure of an **application for a project** to be authorised, in summary requiring that Member States shall ensure that an application for project authorisation is submitted by the user or the person responsible for the project. The application shall include at least the following: (a) the project **proposal**; (b) a **non-technical project summary** (this may not be required by Member States for project applications under Article 42); and (c) information on the elements set out in Annex VI, and the elements are:

- **Relevance and justification** of the following: use of animals including their origin, estimated numbers, species and life stages; and procedures.

- Application of **methods to replace, reduce and refine** the use of animals in procedures.

- The planned use of anaesthesia, analgesia and other pain-relieving methods.

- Reduction, avoidance and alleviation of any form of animal suffering, from birth to death where appropriate.

- Use of humane endpoints.

- Experimental or observational strategy and statistical design to minimise animal numbers, pain, suffering, distress and environmental impact where appropriate.

- Reuse of animals and the accumulative effect thereof on the animals.

- The proposed **severity classification** of procedures (Defined and detailed in Annex VIII section 1 as being non-recovery, mild, moderate and severe).

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[39] National authorities are listed at https://ec.europa.eu/environment/chemicals/lab_animals/ms_en.htm

Discussion point: Explore what the severity classifications mean. Take a look at the [Severity Assessment Framework](#) from the European Commission. Download the Severity Assessment Framework Poster.

- Avoidance of unjustified duplication of procedures where appropriate.

- Housing, husbandry and care conditions for the animals (Which is defined and detailed in Annex III).

- Methods of killing (With approved methods and protocols being defined and detailed in Annex IV).

- Competence of persons involved in the project.

Applications will be evaluated by the competent authority (Within 40 days[40] of receiving an application and '*subject to safeguarding intellectual property and confidential information, the project evaluation shall be performed in an impartial manner and may integrate the opinion of independent parties*') using the following key criteria:

- The project is justified from a **scientific or educational** point of view or required by law.

- The purposes of the project **justify the use of animals**.

- The project is designed so as to enable procedures to be carried out in the most **humane and environmentally sensitive** manner possible.

Article 13 details the specific means of selecting a choice of procedures that

- Use the minimum number of animals.

- Involve animals with the lowest capacity to experience pain, suffering, distress or lasting harm.

- Cause the least pain, suffering, distress or lasting harm.

- Are most likely to provide satisfactory results.

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[40] '*When justified by the complexity or the multi-disciplinary nature of the project, the competent authority may extend the period referred to in paragraph 1 once, by an additional period not exceeding 15 working days.*' And '*In the case of an incomplete or incorrect application, the competent authority shall, as quickly as possible, inform the applicant of the need to supply any additional documentation and of any possible effects on the running of the applicable time period.*'

- And, avoid the death of an animal wherever possible, or use humane end-points.

The evaluation process will involve:

- An evaluation of the **objectives** of the project, the predicted scientific benefits or educational value.

- An assessment of the **compliance** of the project with the requirement of replacement, reduction and refinement.

- An assessment and assignment of the classification of the **severity** of procedures.

- A **harm-benefit analysis** of the project, to assess whether the harm to the animals in terms of suffering, pain and distress is justified by the expected outcome taking into account ethical considerations, and may ultimately benefit human beings, animals or the environment.

- An assessment of any **justification** referred to in articles 6 to 12 (the killing of animals and particular conditions for some animals), 14 (anaesthesia), 16 (Reuse of animals) and 33 (Care and accommodation of animals).

- A determination as to whether and when the project should be **assessed retrospectively**.

The evaluation will assess the **expertise** of the applicants, relating to:

- The **areas of scientific use** for which animals will be used including replacement, reduction and refinement in the respective areas.

- **Experimental design**, including statistics where appropriate.

- **Veterinary practice** in laboratory animal science or wildlife veterinary practice where appropriate.

- **Animal husbandry and care**, in relation to the species that are intended to be used.

If the evaluation is successful then the competent authority can grant a **project authorisation, for a period not exceeding 5 years.** The authorisation will confirm that it is limited only to the procedures (and their severity levels) detailed in the application. It will specify who is the **user** ('*any natural or legal person using animals in procedures, whether for profit or not*') undertaking the project, the **persons who will be '***responsible for the overall implementation of the project and its compliance with the project authorisation*', the **establishment**(s) in which the project will be undertaken, and '**any specific conditions** following the project evaluation, including whether and when the project shall be assessed retrospectively.' In addition:

> '*Member States may allow the authorisation of **multiple generic projects** carried out by the same user if such projects are to satisfy regulatory requirements or if such projects use animals for production or diagnostic purposes with established methods.*'

Download the [Project Evaluation and Retrospective assessment Report](#)

Download the Project Evaluation Poster.

Optimise the tests on animals: PREPARE before you ARRIVE - Slide 20

There are two methodologies that can help projects to address the **Three Rs** at the stages of being prepared and reported. If project proposals are to be well-structured, high quality and complete, they need clear guidance and support. They also need the same structure and rigour to report their outcomes.

The first is **PREPARE**[41] (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence). There is a **structured checklist**[42] for proposers to go through, with the aim of ensuring that they have addressed every element of a complete application. The checklist is structured under three key headings, with 15 sub-themes (and then more detailed elements to 'check' off in each of the sub-themes). Heading and sub-themes are:

- **Formulating the proposal**, applicants should cover: 1. Literature searches; 2. Legal issues; 3. Ethical issues, Harm-Benefit Assessment and humane endpoints; 4. Experimental design and statistical analysis.

- A **dialogue should occur between the scientists and the animal facility** relating to: 5. Objectives and timescale, funding and division of labour; 6. Facility evaluation; 7. Education and training; 8. Health risks, waste disposal and decontamination.

- **Quality control of the components of the project** should cover: 9. Test substances and procedures; 10. Experimental animals; 11. Quarantine and health monitoring; 12. Housing and husbandry; 13. Experimental procedures; 14. Humane killing, release, re-use or re-homing; 15. Necropsy.

A wide range of resources are available on the PREPARE website. For literature searches it is important that a search is as complete as possible, so that e.g. unnecessary duplication can be avoided. It is advisable to do an extensive search with help from specialised staff at university / medical libraries.

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[41] https://norecopa.no/prepare
[42] https://norecopa.no/prepare/prepare-checklist

The second set of resources are the **ARRIVE**[43] guidelines for the reporting of *in vivo* projects. The structured guidelines[44] have five main themes and 20 sub-themes (many with more detailed elements) covering:

- **Title** of the project and **abstract** of the report material.

- Introduction: **background and objectives**.

- **Methods**: ethical statement, study design, experimental procedures, experimental animals, housing and husbandry, sample size, allocating animals to experimental groups, experimental outcomes, statistical methods.

- **Results**: baseline data, numbers analysed, outcomes and estimation, adverse events.

- **Discussion**: interpretation and scientific implications, generalisability and translation (to other species or systems), funding sources.

Discussion / activity: Using two groups of students ask one group to make a mock proposal for a project. The other group represents the ethics committee that would accept or reject their request.

Roles and procedures under Directive 2010/63/EU - Slides 21 & 22

Article 24 of the Directive itemises '**specific requirements for personnel**':

"*Member States shall ensure that each breeder, supplier and user has one or several persons on site who shall: be **responsible for overseeing the welfare and care** of the animals in the establishment; ensure that the staff dealing with animals have **access to information specific to the species** housed in the establishment; be responsible for ensuring that the **staff are adequately educated**, competent and continuously trained and that they are supervised until they have demonstrated the requisite competence*."

The people who are specified in the Directive must: '*ensure that any unnecessary pain, suffering, distress or lasting harm that is being inflicted on an animal in the course of a procedure is stopped; and ensure that the projects are carried out in accordance with the project authorisation or, in the cases referred to in Article 42, in accordance with the application sent to the competent authority or any decision taken by the competent authority, and ensure that in the event of non-compliance, the appropriate measures to rectify it are taken and recorded.*'

Article 25 defines the need for a **designated veterinarian**, and '*Member States shall ensure that each breeder, supplier and user has a designated veterinarian with **expertise in laboratory animal medicine**, or a suitably qualified expert where more appropriate, charged with advisory duties in relation to the well-being and treatment of the animals.*'

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[43] https://www.nc3rs.org.uk/arrive-guidelines

[44] https://www.nc3rs.org.uk/sites/default/files/documents/Guidelines/NC3Rs%20ARRIVE%20Guidelines%202013.pdf

Discussion point: How can a veterinary qualification from one Member State be recognised in another? It invites a discussion about mobility in the Single Market, and about the mutual recognition of qualifications – a major policy initiative of DG Employment[45]. In addition, the European Council for Laboratory Animal Medicine provides a specialty training for veterinarians across Europe, which is acknowledged as a veterinary specialty by the European Board for Veterinary Specialisation.

Article 34 requires Member States to '*ensure that the competent authorities carry **out regular inspections of all breeders, suppliers and users, including their establishments**, to verify compliance with the requirements of this Directive.*' The **frequency of inspections** should be undertaken on the **basis of a risk analysis for each establishment**, rather than on a fixed time period. The risk analysis should take account of: '*the number and species of animals housed; the record of the breeder, supplier or user in complying with the requirements of this Directive; the number and types of projects carried out by the user in question; and any information that might indicate non-compliance*.'

Discussion point: What is the difference between a regular programme of inspections and a programme based on risk analysis?

Member States must inspect at least **one-third of the users each year**, with the exception that '*breeders, suppliers and users of non-human primates shall be inspected at least once a year.*' There is a requirement that '*an appropriate proportion of the inspections shall be carried out without prior warning. Records of all inspections shall be kept for at least 5 years*.'

Article 35 relates to '***controls of Member State inspections***', noting that while the Member States are responsible for carrying out inspections, the Commission also needs to be confident that the inspections are being carried out in accordance to the Directive.

The Commission may have '*due reason for concern, taking into account, inter alia, the proportion of inspections carried out without prior warning, undertake controls of the infrastructure and operation of national inspections in Member States*.'

In such circumstances the Member State must assist the experts who are appointed by the Commission to carry out this action, and they will inform the Commission of their recommendations, and the Commission will inform the '*competent authority of the Member State … [which] shall take measures to take account of the results of the control.*'

Discussion point: Does this process ensure that there is independent oversight of the Member State inspections?

Non-technical project summary – Article 43

Article 43 requires a **non-technical project summary** to be prepared and submitted with the project proposal. It should describe the project in a way the lay person can understand, including information on the objectives of the project and how the Three Rs have been

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[45] See https://www.fve.org/eccvt/

applied, and specify whether a retrospective assessment is needed. It should be made publicly available. **Decision 2020/569 on Reporting** provides a mandatory template for non-technical project summaries. In 2021 the Commission will provide a searchable, public database where all the NTS can be found.

For projects that are approved, Article 39 concerns **'retrospective assessment'**, which means that once a project has been carried out, it needs to be evaluated. All projects that have specified in their application that they will use non-human primates, and all those which will involve processes classified as severe, must be retrospectively assessed. Member States may exempt from assessment projects where procedures are classified as 'mild' or 'non-recovery'.

The Directive notes the need to **develop a coherent approach to evaluation and project review** across Member States, and to develop coherence it requires Member States to establish **National Committees**[46] '… *to give advice to the competent authorities and **animal-welfare bodies** in order to promote the principles of replacement, reduction and refinement*.' The National Committees form a network which exchanges knowledge and best practice – in July 2019 it produced *guidance to facilitate the implementation of Directive 2010/63/EU (article 49)*.[47]

Download the [Animal Welfare Body and National Committees Report](#)

Download the Animal Welfare Body Poster

4. Directive 2010/63/EU and the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)

European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) - Slide 23

EURL ECVAM coordinates many activities supporting the implementation of the Three Rs and validation. International cooperation, education, advisory committees and networks towards validation in regulatory networks are part of its focus, in order to work towards development, validation and regulatory use of alternatives worldwide[48].

Articles 48 and Annex VII of Directive 2010/63/EU set out the duties of the **European Union Reference Laboratory for alternatives to animal testing** (known as EURL ECVAM) at the European Commission's Joint Research Centre, in Ispra, Italy.

The Directive 2010/63/EU anchored a formal legal base for EURL ECVAM, although it had already existed since 1991.

The tasks and duties include:

- conducting research and collaborating in EU and international research initiatives;

- coordination and undertaking of validation studies of alternative methods for the safety assessment of chemicals;

- dissemination of information and sharing of knowledge across disciplines and sectors; and

- promotion of alternative methods and the Three Rs in an international context.

  Discussion point: What are the activities at EURL ECVAM *that can help in our teaching, learning, and research? (Look at the range of activities such as information resources - some of these are explored in more detail in later slides).*

  Discussion point: What is validation? Why is it important? What is the value of regulatory agencies being involved in a validation process?

Implementation at Member State level – the role of national contact points, committees and the PARERE network - Slide 24

Article 48 of the Directive establishes the EURL ECVAM Reference Laboratory, and Annex VII (details the duties of EURL ECVAM) one of the duties is:

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[48] https://ec.europa.eu/jrc/en/eurl/ecvam

> "*promoting dialogue between legislators, regulators, and all relevant stakeholders, in particular, industry, biomedical scientists, consumer organisations and animal-welfare groups, with a view to the development, validation, regulatory acceptance, international recognition, and application of alternative approaches.*"

This is undertaken through the **PARERE (Preliminary Assessment of Regulatory Relevance) Network**[49]. Member States must identify a single point of contact to PARERE to provide advice on the regulatory relevance of alternative approaches proposed for validation.

The PARERE network meets formally once each year, but has regular interaction with members[50], where '*requests, input and collaborative activities are addressed through written procedure and an internet-based forum for information exchange.*'

> *Discussion point: ask students to take the latest meeting report[51] and to review the key discussion points. Students could re-enact this meeting, or hold their own PARERE meeting.*

Implementation at Member State level – Validating alternatives through EU-NETVAL - Slide 25

EU-NETVAL (European Union Network of Laboratories for the Validation of Alternative Methods) is a network of EU Member State laboratories and was set up by EURL ECVAM in response to the provision of Directive 2010/63/EU (Art 47) which requests that EU Member States assist the European Commission in the validation of alternative methods.

Member States are encouraged to nominate specialised laboratories to support EURL ECVAM's alternative method validation studies, and currently there are 35 member labs.

Members are selected against pre-defined eligibility criteria and endorsed by the National Contact Points for the Directive

EU-NETVAL aims to support validation studies undertaken by EURL ECVAM to assess the reliability and relevance of alternative methods that have a potential to replace, reduce or refine the use of animals for scientific purposes.

More information about EU-NETVAL can be found here:

> *Discussion point: Has my Member State nominated a lab to EU-NETVAL? What could be the reason a Member State nominates or does not nominate a specialised laboratory to contribute to development and validation of alternative methods.*

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[49] https://ec.europa.eu/jrc/en/eurl/ecvam/alternative-methods-toxicity-testing/advisory-bodies/parere
[50] The current list of members is at https://ec.europa.eu/environment/chemicals/lab_animals/parere_en.htm
[51] https://ec.europa.eu/jrc/sites/jrcsh/files/summary_record_parere_meeting_2019.pdf

Annex – Glossary of terms

Acronym	Meaning
ALTEX	Alternatives to Animal Experimentation (an open access journal)
ARRIVE	Structured guidelines for the reporting of in vivo projects
CRISPR	Clustered regularly interspaced short palindromic repeats (a family of DNA sequences). CRISPR is a gene editing technology.
DA	Defined Approach
DNA	Deoxyribonucleic acid (a molecule that encodes an organism's genetic blueprint)
EC	European Commission
ECVAM	European Union Reference Laboratory for Alternatives to Animal Testing
EDA	The Experimental Design Assistant
EEC	European Economic Community
EFSA	European Food Safety Authority
EP	European Parliament
EU	European Union
EU-NETVAL	European Union Network of Laboratories for the Validation of Alternative Methods
EURL ECVAM	European Union Reference Laboratory for Alternatives to Animal Testing
Ex vivo	Experimental techniques using organs and tissues taken from an organism
In chemico	Experimental techniques using biochemical approaches
In silico	Experimental technique using computer modelling or computer simulation
In vitro	'In glass', or performing a procedure outside of a living organism, for example in a test tube or culture dish
In vivo	Performing a procedure using a whole, living organism
IATA	Integrated Approaches to Testing and Assessment

ICT	Information Communication Technologies
JRC	Joint Research Centre of the European Commission
LD	Lethal Dose
MAD	Mutual Acceptance of Data
MEP	Member of the European Parliament
MOOC	Massive Open Online Course
NC3Rs	National Centre for the Replacement Refinement & Reduction of Animals in Research
NORECOPA	Norway's Three R centre and National Consensus Platform for the Replacement, Reduction and Refinement of animal experiments
OECD	Organisation for Economic Co-operation and Development
PARERE	Preliminary Assessment of Regulatory Relevance Network
PBK/D	Physiologically Based Kinetic and Dynamic models
QSAR	Quantitative Structure-Activity Relationship
SYRCLE	Systematic Review Center for Laboratory animal Experimentation
TED talk	TED (technology, entertainment, design)
TFEU	Treaty on the Functioning of the European Union
Three Rs	Replacement, Reduction, and Refinement of animal use in science
TSAR	Tracking System for Alternative methods towards Regulatory acceptance
Validation (to validate)	The process of confirming that a test method is suitable for its intended purpose through official channels.

Setting the Scene – understanding the different positions regarding the use of animals for scientific purposes

Before using the material that supports the slides, this section aims to give some perspectives to consider. They are presented in no order of importance.

> Discussions about the use of animals in science should start by acknowledging the various positions, ranging from those who support it as being the 'gold standard' in protecting humans from harm, to those who want to ban animal testing as a matter of principle. How you start also depends on the disciplinary context within which you are using this material. For example:
>
> - An ethics context may start from a position defending 'animal rights';
>
> - A bioscience and medical perspective may start by developing the arguments from the context of what animal testing has achieved, while acknowledging that testing can involve inflicting harm and suffering on animals;
>
> - A technology perspective may look ahead at the opportunities that genetics, big data, and the synthesising of human organs can help us to move away from the use of animals;
>
> - A social studies perspective could ask the challenging questions about using animals as food, pets, sources of entertainment, and set that in the context of scientific experimentation using animals.

The passionate argument to stop using animals for scientific purposes

One of the more passionate arguments against animal testing is presented by Dr. Charu Chandrasekera in her TED talk "*It's time to think outside the cage*"[52]. She argues that the translation from animal studies to humans is largely insufficient, and that replacement alternatives based on the use of human materials are the way forward to improve the situation. She notes some of the scientific innovations that can help to move away from using animals, and some her main points (with additional context) are:

- We have found cures for contemporary illnesses such as Parkinson's, Alzheimer's, Heart Failure, Cystic Fibrosis – but we cured them in mice, not humans.

- We have had to withdraw some drugs from use because, while they were tested as being safe in animals, they were not safe for humans.

- It is true that over the last 100 years there are many examples of important medicines being developed as a result of animal experiments. However, for every

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[52] http://www.uwindsor.ca/ccaam/

success there are multiple failures and a high percentage (95%[53]) of animal experiments do not lead to a successful medicine coming to market. In addition, of the 5% of compounds that successfully make it through animal trials almost a half are subsequently withdrawn because they show adverse effects in humans.

- There are strong social pressures[54] to change the paradigm that testing on animals is a socially acceptable and scientifically reliable surrogate for humans.

- She cites the case of the drug Vioxx[55], passed testing on animals and clinical trials on humans and was introduced in 1999 as an anti-inflammatory drug for the treatment of arthritis. There were no adverse cardio-vascular problems in the animals, and in mice it was shown that it could be beneficial in that context. After more long-term use in humans, Vioxx use became associated with an increased risk of coronary heart disease. In the USA, it was estimated that 88,000 to 139,000 Americans had heart attacks and strokes which seemed the result of taking the drug[56].

- Mice and rats closely emulate our biology (and represent about 90% of all animals used in testing), and they have short life spans. However, all animal species are 'separated' from humans and each other by having different genetic, physiological, biochemical factors. Toxicological studies using rats and mice showed similar responses in only 60% of cases so it is not surprising that rat and mice models are very often different to humans. These factors are complicated by variability in age and sex and other factors.

- Taking the case of Alzheimer's disease, diagnosed first in 1906[57]. After extensive research it can be cured in mice, but not yet in humans. Over 400 clinical trials have failed[58].

(*Discussion point – a 99.6% failure rate of drugs entering the market has been claimed – consider the economic and social cost of this. Is this statistic really accurate? Develop a debate on the use of statistics and evidence, for example using the examples provided by Chris Magee, such as:*

"It is worth noting that around 90% of drugs are removed at every stage of safety tests, i.e. 90% are removed at non-animal pre-clinical safety tests, 90% at animal stages, and 90% during human clinical trials. In fact, working backwards, if we have 1000 drugs entering animal safety tests, 900 of them fail, of which 20 might be safe in humans. Of the remaining 100, 92 fail human tests, therefore: 90.5% of dangerous drugs have been kept out of clinical trials thanks to animal safety tests"[59])

- Given the advances in gene-technology, bio-technology and computer science, is now the time to move on from a scientific culture based on animal models, especially since many of the major diseases (cancer, diabetes, stroke etc.) have

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[53] Statistics are controversial – see the later material in the storyboard
[54] Here you can explore the growth of animal rights groups such as PETA https://www.peta.org/
[55] https://www.bmj.com/content/334/7585/120
[56] https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(05)17864-7/fulltext
[57] https://www.alzheimers.net/history-of-alzheimers/
[58] Back in 2014 this issue was already being discussed https://doi.org/10.3389/fphar.2014.00146
[59] http://www.understandinganimalresearch.org.uk/news/communications-media/more-myths-busted/

not seen enough effective cures emerging from animal-based research. Should we refocus our attention whether modern non-animal approaches can accelerate medical advances and eradicate some of the failures of animal research?

- Since the mapping of the human genome was completed in 2003, we have new knowledge and tools to research human biology. We can use cells and tissues from dead bodies and surgical remains, and we can now engineer cells artificially. A small biopsy from your skin can be used to generate adult stem cells[60], and for those cells to be 're-programmed' to become any cell type in your body. Those cells can be taken and engineered to form more complex structures such as Organoids (small organs). Stem cell manipulation and 3D bioprinting[61] may produce more representative disease models than animals - the so-called 'disease on a dish' or 'toxicity on a chip'[62] may be more useful models against which to test drugs.

- Other new tools include non-invasive imaging, and computational modelling (advanced computational power, big data, and artificial intelligence[63]) to analyse the vast array of data gathered from experiments in the past. For example, this approach is generating much more insight into Alzheimer's disease than has all the previous animal research. Stem cells can be taken from human patients who have died of the disease and used to grow brain cells that carry the disease within them. These cells can be assembled into structures called 'mini-brains[64]. 'Parkinson's in a dish[65]' is a similar approach to finding treatments for Parkinson's Disease.

- By replacing animals with validated alternatives the effects of novel compounds can be more accurately predicted. In some instances, computer models have been shown to be more successful at predicting toxicity in humans than previous animal tests[66].

(*Discussion point: High quality science is needed, also for replacement alternatives and human relevant science. This is an opportunity to consider what is 'good science[67]' in the European context*).

- By replacing animal testing, we may have the potential to save some costs, develop solutions faster, reduce the failure rates (computer simulations, (that might be based on existing data from animal studies), and possibly supported by other approaches, can be more successful at assessing toxicity than performing new animal tests), making the research more human-relevant, and being more humane in our treatment of animals.

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[60] Different types of stem cells and their sources: https://www.researchgate.net/figure/Different-types-of-stem-cells-and-their-sources-ESCs-embryonic-stem-cells-iPSCs_fig1_333878331
[61] Some background can be found here: https://www.youtube.com/watch?v=i8uCs09BoNU
[62] A possible point to stop and discuss 'what are these processes' – ask the learners to explore material and provide simple summaries
[63] An opportunity to have a discussion on big data and artificial intelligence in biomedical research
[64] https://www.sciencedaily.com/releases/2019/06/190627113945.htm
[65] https://www.jhsph.edu/news/news-releases/2016/researchers-create-mini-brains-in-lab-to-study-neurological-diseases.html
[66] https://www.youtube.com/watch?v=VcrknGTZxyY and
https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2019.e170710
[67] https://ec.europa.eu/research/infocentre/article_en.cfm?id=/research/headlines/news/article_18_08_07-1_en.html?infocentre&item=Infocentre&artid=49608

- Furthermore, there is enormous potential for 'personalising medicine'. A small biopsy of your skin could be used to create your own mini-organs (mini-brains, organs-on-a-chip), on which hundreds of drug compounds are tested so that the best drug for you and your condition is selected.

The role of animals in scientific research

We are not yet able to fully replace animal use in science, but significant advances are being made. There is significant political momentum in countries across the world to seek alternative methods to using animals. Alternative methods help to overcome the ethical issues of using animals (*see the slides discussing ethics, and regarding animals as 'sentient' beings*).

There are some helpful articles and videos of lectures covering how animal experimentation has contributed to the development of medicines[68]. There are also useful teaching resources which provide an overview of animal research[69].

There is growing evidence that advances in genomics, stem cell science, computer modelling and computational science, systems biology and the like may mean that animal use in science will become a thing of the past. Aligned to the work going on to validate various alternative methods there is a strong scientific argument for replacement.

However, that is the future, whatever our concerns about animals and suffering. The tension in the debate is about the speed at which we reach full replacement, and that depends very much on the levels of investment that are made into the new approaches, the training of new scientists who can take the research forward (hence the importance of introducing this material into the school curricula), how business and industry sees the commercial advantage of moving to new approaches, and the extent to which societal pressure influences changes in policy.

These issues are important discussion points as you use the following material in your teaching and learning contexts, noting also that your students may be able to 'learn' about the physiology of animals without dissecting them – for example using simulations and 3-D models[70]. Some other resources you could consider are:

- A four-minute introduction to the Three Rs[71].

- A series of presentations "Improving openness in animal research in Germany"[72].

- No more lab rats - Thomas Hartung. A 15-minute presentation on the future of toxicity testing[73].

For the present, we are on a journey towards replacing animal testing. The pharmaceutical industry still needs by law to test potential new medicines on animals before they can go into clinical trials, and to date there are no 'alternatives' available to the industry for that

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[68] http://www.animalresearch.info/en/
[69] http://www.understandinganimalresearch.org.uk/teacherszone/teaching-ideas-and-resources/
[70] Examples are provided at https://www.forhumanescience.org/what_we_do/influencing-science-culture/humanescienceeducation_schools/teacher-portal/
[71] https://www.youtube.com/watch?v=onqmtKnNsmY
[72] http://eara.eu/en/improving-openness-in-animal-research-in-germany-watch-the-videos/
[73] https://www.youtube.com/watch?v=VcrknGTZxyY

process and no examples of new potential medicines being tried in humans just on the strength of efficacy and safety being demonstrated in non-animal models.

Animals are also still important in some areas for example, education and training in higher education: undergraduate, postgraduate, post-doctoral and medical. Directive 2010/63/EU therefore aims to help accelerate the move to replacement, while ensuring that rigorous conditions are in place (to reduce and refine) where animal use for scientific purposes is still accepted as being justified.

Examples of teaching and learning journeys

Some example teaching and learning journeys could be:

- Biology/biosciences: focusing on the more advanced science to move away from animal testing;

- Information technology: the opportunities and challenges involved in using new computational techniques and big data;

- Politics: the ways in which social attitudes to animal testing have influenced policy developments. The 'contested' nature of statistics and evidence in the often emotional debates about animal testing;

- European Studies: building an understanding of how the European Union works in bringing together the Member States to work towards replacing animal use in science;

- Social studies and ethics: exploring the ethical and societal issues in our relationships with animals;

- History of science: looking at how scientific paradigms emerge, for example the relationship between science, policy, and legislation;

- Business studies: the business opportunities that exist in new medical and health technologies;

- Law: the development and implementation of European Union legislation.

The historical and social background is presented first, followed by the comprehensive procedures and support structures that are provided within the EU Directive. The Three Rs principles are described in more detail, and then some of the scientific innovations that are helping to move towards replacing animal testing in the European Union are explored.

For example, another way you can structure the material could be:

Section 1. European Legislation relating to animal experimentation

- Brief history of animal use

- Ethics and societal views and how they have changed – opportunity to introduce the Three Rs concept

- Legal requirements relating to mandatory animal testing in order to bring a new medicine to the market

- How many animals are used for scientific purposes now (In the EU?) with pertinent stats.

Section 2. Animal use in science

- Distinguish between animals used for:
  - o Medical research

- Scientific research – better understanding of anatomy, physiology, pharmacology, biochemistry etc

- Education and Training

- Cosmetics, food and hygiene testing

- Chemical safety testing

- Compare animals used for food, as pets, for entertainment (e.g. circuses, zoos, bull fighting) etc.

Section 3. Methods to support the Three Rs now and in the future

- Replacement – the ultimate goal
  - New scientific innovations and what they mean for animal experimentation
    - New 'human' preparations e.g. organs-on-a-chip, mini brains
    - Advanced computing – better interrogation (big data techniques, artificial intelligence) of existing data about putative compounds (chemical libraries), data from completed animal experiments, existing knowledge of disease
    - Systems biology approaches

- Reduction
  - Selecting the most appropriate animal model – better understanding of species differences (comprehensive literature searching) and how they respond to different drugs to enable human disease to be better modelled;
  - Better experimental design and using appropriate statistics

- Refinement
  - Reducing animal stress through better housing, handling and treatment

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