European Commission

Introducing the Three Rs into secondary schools, universities and continuing education programmes

Replacement, Reduction and Refinement of animal use in science

Joint Research Centre

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Introducing the Three Rs into secondary schools, universities and continuing education programmes

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1 07 02 77 41. Pilot project — Promoting alternatives to animal testing

Executive summary

This report targets **decision-makers and influencers within education systems** who contribute to policy-making at European, Member State or local level, and who stimulate and facilitate the development and uptake of new educational content and resources.

According to the latest EU statistics 9.39 million animals were used in research and testing in 2017. A similar number of laboratory animals were bred for science but never actually used. Although in recent years the numbers have declined, there is a long way to go before the EU's goal of eliminating animal-use in science is reached.

9.39 million
is the number of animals used in research and testing in EU in 2017

In the European Union, Directive 2010/63/EU on the protection of animals used for scientific purposes fully embraces the **principles of the Three Rs**, to Replace, Reduce and Refine the use of animals for experimental or educational purposes.

The European Commission's Joint Research Centre (JRC) undertakes a number of initiatives to improve knowledge sharing on the Three Rs to encourage their uptake and implementation.

In 2016 the JRC conducted a study to identify existing Three Rs knowledge sources and to understand how they are used and shared (Holley *et al.*, 2016). One of the key findings was that Three Rs knowledge sharing could be enhanced through the development of **targeted education and training**

initiatives and resources, and a subsequent study was conducted to map and characterise the already existing ones worldwide. A main conclusion was that, although Three Rs courses and resources are abundant, there is a need to further complement and amplify current education and training opportunities, both at the level of secondary school and higher education. Another related study undertaken focused on how to **incorporate the Three Rs** and related teaching material **into the curriculum** of schools and universities.

This report is based on the knowledge and input gathered from a variety of expert sources and intends not only to provide the **'business case' for introducing the Three Rs** into curricula and education programmes, but also suggests how this can be achieved using **different means**. It includes examples of learning scenarios and teaching resources to both inspire and facilitate action.

Next steps for this important initiative will involve further outreach to educators, experts and education decision-makers, providing support and training for Three Rs teachers, and highlighting new career and job opportunities stemming from the significant impact of the Three Rs in many different sectors.

More effective and efficient teaching requires guidance and resources such as: ready-to-use lesson plans, course structures and innovative classroom approaches. Whilst the JRC will continue its own efforts in this area, a combined and coordinated effort by educators and Three Rs specialists will deliver more impactful results of significant benefit to students across the EU.

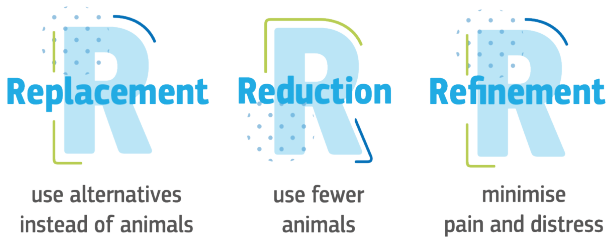
1. Introduction

The Three Rs (Replacement, Reduction, Refinement of animal use in science, (see Box 1) were first put forward by Russell and Burch in 1959 with the publication of "The Principles of Humane Experimental Technique".

The Three Rs principles have stood the test of time and not only provide a sound framework for performing humane animal research but also offer compelling education opportunities covering a variety of relevant topics and perspectives.

Box 1. The Three Rs

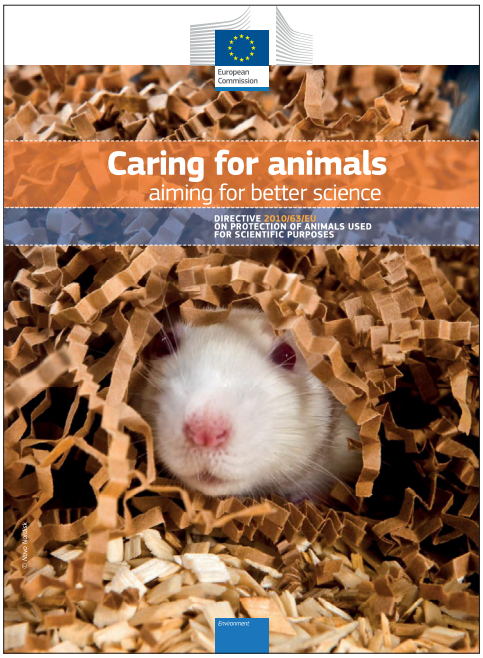
Replacement is defined as methods, strategies or approaches, which do not involve the use of live animals, for example *in vitro* systems using tissues or cells. **Reduction** refers to any approach that results in fewer animals being used to achieve the same objective, including maximising the information 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 procedure 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.



1.1 The Three Rs and EU legislation

Animal welfare is a European value and is embedded in the Treaty on the Functioning of the European Union. As set out in Article 13 of the Treaty (EC, 2012), the welfare of animals must be taken into account in the Union's policy on internal market, research and agriculture, among others.

Directive 2010/63/EU (EU, 2010) on the protection of animals used for scientific purposes explicitly recognises and embraces the Three Rs. The Three Rs principles must be applied in all aspects of animal use, not only in scientific procedures but also in relation to their breeding, housing and care. Although the Directive acknowledges that animals still need to be used in science where non-animal alternatives are lacking, the **ultimate aim of EU policy** is focused on one 'R', full **Replacement**.



As a cross-cutting piece of legislation, the Directive requires the application of the Three Rs in all major legal acts of the European Union that may require animal use for scientific purposes. Thus, for example, legislation to ensure the safe use of chemicals in a variety of sectors including plant protection products (e.g. pesticides), biocides (e.g. disinfectants) and food explicitly require that the toxicological information requested be generated using non-animal testing methods where possible. 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.

Although the Directive establishes requirements for the education, training and level of competence of personnel that care for animals and who design and conduct procedures and projects using animals, it does not cover the introduction of the Three Rs in general education. Therefore, the choice to introduce the Three Rs into curricula and courses offered by schools, universities and continuing education programmes lies very much with EU Member State institutions and educators.

1.2 Attitudes and ethics

Animal welfare is an important issue for many European citizens. According to a special **Eurobarometer survey**[1], the majority of Europeans (87%) consider information campaigns on animal welfare to be a good way to influence the attitudes of children and the younger generation towards animals.

1 *https://ec.europa.eu/commfrontoffice/publicopinion/index.cfm/ResultDoc/download/DocumentKy/71348*

Stop Vivisection[2], brought forward as an EU Citizens' Initiative, collected 1.2 million signatures across 26 Member States and called for a complete phasing out of animal experimentation. The motivation was based both on animal welfare considerations but also on the importance of using human relevant models to safeguard human health.

Recently, members of the European Parliament have called for a **worldwide ban on testing cosmetics on animals**[3], based on the EU experience with its own ban which has been in force since 2013.

The majority of published contemporary biomedical research involving the use of live animals has been evaluated carefully to ensure that appropriate ethical and animal welfare standards are met. EU Directive 2010/63/EU reinforces the idea that animal experiments must be based on ethical considerations and provides a legal framework to achieve this.

The approach to the use of animals in science in accordance with this framework starts with European attitudes towards animal welfare enshrined in the Treaty, leading to the statement in the Directive itself that says: '*Animals have an intrinsic value which must be respected.* [and that they] ... *should always be treated as sentient creatures*'. The practical approach set out by the Directive stems naturally from this statement.

A number of measures facilitate this, for example:

- the nurturing of a 'climate of care' (also referred to as 'culture of care') in each establishment using animals (supported by the respective Animal Welfare Body);
- promoting transparency through annual detailed statistics on animal use, publicly available non-technical project summaries and retrospective assessments of completed projects; and
- careful assessment and application of a severity classification for procedures to be performed on live animals (mild, moderate, severe or non-recovery).

However, perhaps most importantly the Directive dictates that no animal may be used in a procedure unless the harms are justified by the expected benefits. Project evaluation assesses this, together with ethical considerations of the proposed use of animals. It forms the core of project authorisation and is designed to address the ethical dilemma or trade-off which exists as long as animals are needed in biomedical research and safety and efficacy testing.

2 *https://europa.eu/citizens-initiative/initiatives/details/2012/000007_en*

3 *http://www.europarl.europa.eu/news/en/press-room/20180426IPR02613/testing-cosmetics-on-animals-meps- call-for-worldwide-ban*

1.3 Innovative science

Animal models have served as a basis for biomedical research, safety testing and development of drugs over several decades. Nevertheless, these models may yield results that cannot always be translated to humans.

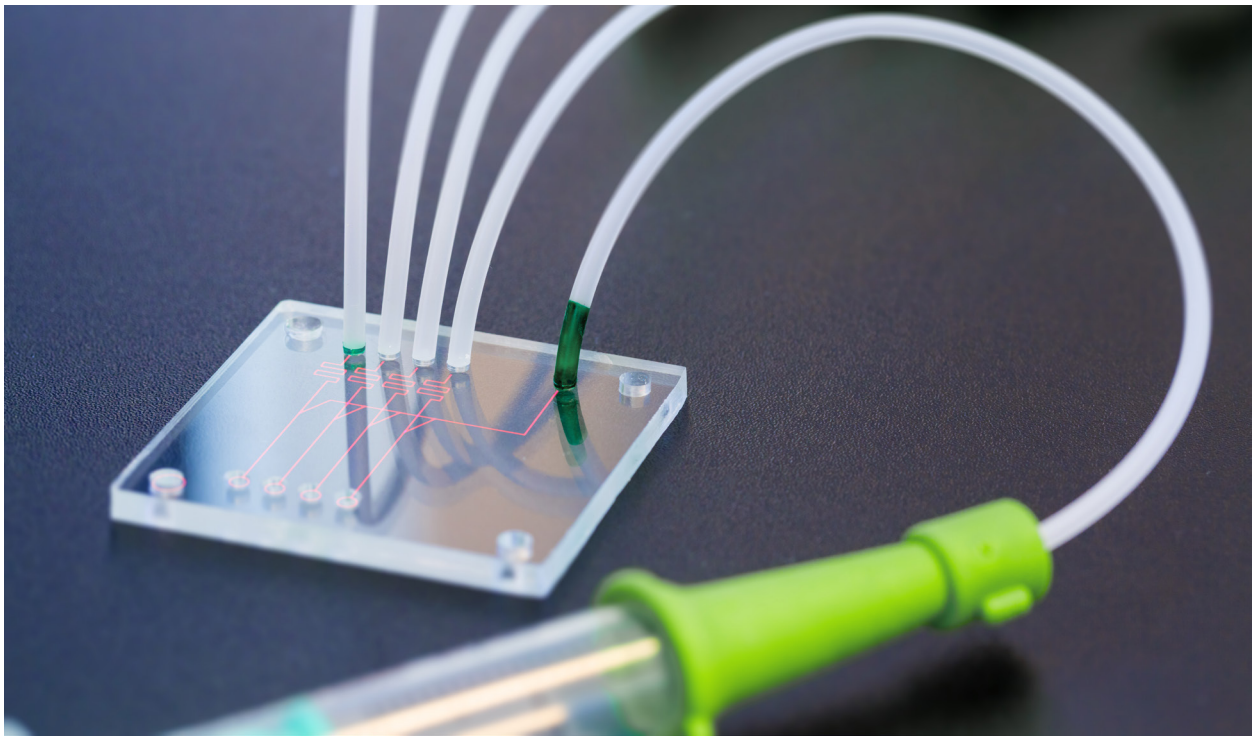
The biology and physiology of rats and mice, the animal species most commonly used in research, differ significantly from that of humans in many respects. Consequently, around 90% of drugs fail in clinical trials, particularly in the area of cancer research (Mak *et al.*, 2014) and Alzheimer's disease. This is, mainly due to unexpected toxicity or lack of efficacy in patients that were not picked up by preclinical animal tests. Therefore, scientific concerns about the predictive power of animal models are driving the development of animal-free, **human-relevant approaches** in different branches of life sciences.

Today, researchers can count on an impressive toolbox of non-animal methods (see Box 2) comprising *in vitro* techniques such as three-dimensional bio-printed tissues, robot-assisted high-throughput screening, high-content imaging, and a suite of 'omics technologies.

Box 2. Non-animal methods

- ***In vitro*** methods: techniques that use cells and tissues cultured in the laboratory
- ***In silico*** methods: computer modelling and simulation
- ***Ex vivo*** methods: cells and tissues explanted from an organism

One of the most important developments in disease modelling has been the generation of **induced pluripotent stem cells** (Takahashi & Yamanaka, 2006) which are functionally equivalent to embryonic stem cells (Kang *et al.*, 2009) and are collectively referred to as pluripotent stem cells. These cells can be derived from patients with genetic disorders and manipulated with genome editing and various differentiation protocols to model pathologies *in vitro*.



Organ-on-chip (OoC) devices, also known as micro-physiological systems, have been shown in recent years to be valuable at multiple stages of the drug discovery and development process. Scientific research employing OoC provides insights into normal human organ function and disease pathophysiology as well as more accurate prediction of the safety and efficacy of investigational drugs in humans (Low *et al.*, 2020). Thus OoC is likely to become a powerful addition to traditional preclinical cell culture methods and *in vivo* animal studies in the short term, and in some cases replacements for them in the longer term (Hay *et al.*, 2014). In the past decade, the OoC field has seen dramatic advances in the sophistication of the underlying biology and engineering, in the demonstration of physiological relevance, and in the range of applications.

Very recently, the JRC's EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) conducted a series of literature reviews on non-animal models in biomedical research. In the area of respiratory tract diseases for example, the review found that more than 200 models have been published in the past five years, ranging from immortalised cell cultures to more sophisticated bioengineering approaches (Hynes *et al.*, 2020). Similar trends can also be seen in breast cancer research (Folgiero *et al.*, 2020).

Computational tools are also widely available for simulating everything from bio-molecular interactions to cardiovascular function, with increasing use of machine learning and artificial intelligence (AI) (Herrmann *et al.*, 2019). Here too, models that are more relevant to human biology will increase our understanding of human disease and will improve the search for effective therapies (Cao *et al.*, 2018).

Scientific innovation that properly addresses societal needs also requires breaking down silos between disciplines and sectors. Disruptive non-animal tools and technologies are helping to **bridge across methods in the biosciences** to facilitate collaboration and knowledge sharing (Carusi *et al.*, 2019).

1.4 Jobs and careers

Recent studies have identified some of the new tools and approaches based on human models as emerging technologies capable of shaping job opportunities of tomorrow[4]. In 2016 the World Economic Forum[5] listed organ-on-chip among the **ten most promising emerging technologies**. Also, data analytics, AI as well as machine learning have been identified as jobs of future in 2022[6].

There is an opportunity to educate students in these new technologies so that they can acquire the necessary expertise to satisfy the increasing demand in related life science fields. This will in turn support them in their pursuit of a 'Three Rs' career for the sake of advancing human-relevant science and reducing the continued reliance on animal models.

There is already strong industry momentum, particularly in the cosmetics sector where animal testing is banned and alternative testing paradigms are being developed. This change in toxicity testing will have a significant impact on the future labour market, which will need specialists knowledgeable in new replacement technologies. Developing alternatives to replace animal experiments is a rapidly growing industrial sector especially amongst small and medium-sized enterprises.

Linking the Three Rs to an innovative, future-relevant and cross-topical educational curriculum will have a significant impact for students and society. Adapting the curriculum to embrace the emerging non-animal paradigm in science will enable the next generation of students to be key enablers in achieving full replacement of animal testing.

4 *https://www.topuniversities.com/student-info/careers-advice/what-can-you-do-biology-degree*

5 *https://www.weforum.org/agenda/2016/06/top-10-emerging-technologies-2016/*

6 *http://reports.weforum.org/future-of-jobs-2018/shareable-infographics/?doing_wp_cron=1566463632.5297069549560546875000*

2. State of play

2.1 Education courses and resources

The JRC has mapped and characterised the provision of **existing courses and resources addressing the Three Rs** across three levels of learning, namely, secondary school, university, and continuing education[7] (Dura & Holloway, 2019). Previous research, which was carried out in the context of Action 1 of the European Commission's response to Stop Vivisection[8], found a clear need for better education to accelerate Three Rs knowledge sharing and uptake (Holley *et al.*, 2016).

The JRC mapping study identified a significant number of courses and resources: 569 in 52 countries worldwide with the majority located in Europe (72%) and North America (16%). In Europe, more than 80 courses are delivered in the United Kingdom, whilst Germany, Switzerland and the Netherlands provide 41, 29 and 26 courses respectively. The teaching language is predominantly English (80%). However, several courses are taught in English and one or more additional languages.

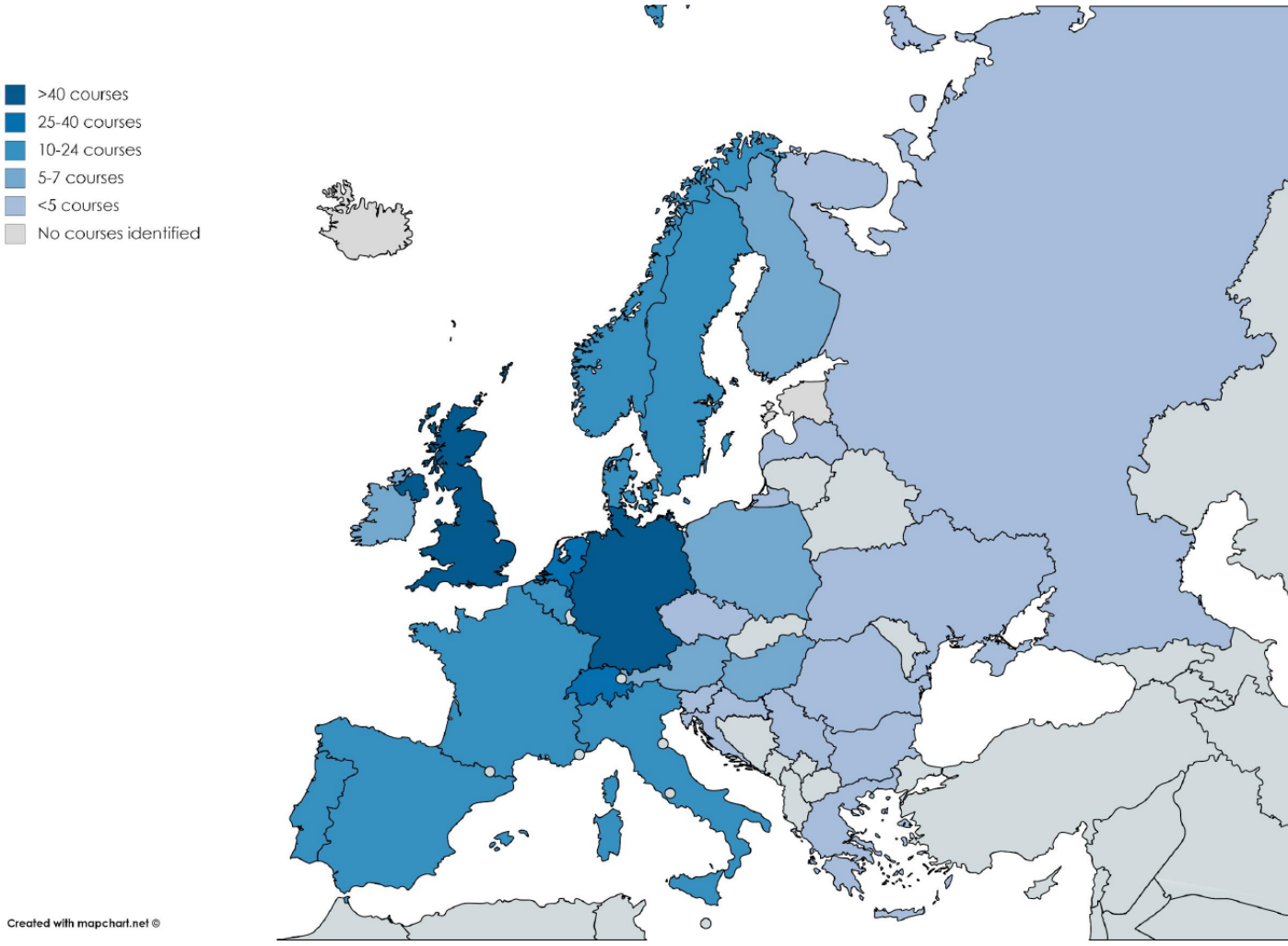
The learning formats offered are usually a combination of both classroom and distance learning. Face-to-face education, in the form of lectures and hands-on training, was identified as the most common format (64%). On the other hand, distance learning through, for example, webinars or interactive online classes represents approximately one third (30%) of the total number of courses and resources.

7 *https://data.jrc.ec.europa.eu/dataset/d0569abb-b5ab-4b9e-9e16-d70cea7a89f9*

8 *https://ec.europa.eu/commission/presscorner/detail/en/IP_15_5094*

Unsurprisingly, the majority of courses and resources (70%) target university level while fewer exist for professionals (26%) and secondary school students (3%). Of the Three Rs, most emphasis was placed on Replacement (32%), followed by Refinement (20%) and then Reduction (8%). Of all the courses and resources identified about one fifth (18%) addressed all of the Three Rs. The Refinement and Reduction principles are usually covered in specific education programmes as mandatory modules for personnel caring for laboratory animals, carrying out procedures and designing projects, as prescribed by article 23 of Directive 2010/63/EU.

Overall, the JRC study showed that there is a significant number of content-rich Three Rs courses and resources available across six continents and accessible in different formats suited to both distance and classroom-based learning. However, certain methodologies and education levels dominate (university level, lecture and practical-based), as does the English language showing an uneven distribution around the Globe. Massive open online courses (MOOCs) and summer schools are under-represented as teaching formats. In addition, **secondary school students** and **professionals** are the least targeted audience.



2.2 An analysis of master courses

University Masters courses identified in the previous study were further explored to analyse their structure, curriculum and how the Three Rs are taught. This study found 94 relevant Masters-level educational programmes distributed mainly in Europe (62%) and North America (30%).

Masters courses were categorised according to their content. Four categories were identified: Laboratory Animal Science, Toxicology, Environment and Ecotoxicology, and Alternative Methods (see Box 3).

Box 3. Masters course programmes related to the Three Rs

- Masters in **Laboratory Animal Science**. The focus is on Refinement and Reduction, teaching topics such as quality of research and wellbeing of the laboratory animals, e.g. pain relief, euthanasia, experimental design, care and nutrition, handling, and giving post-operative care. In some cases, at the end of the programme, the participants get a certificate recognised by the Federation of European Laboratory Animal Science Associations (FELASA).
- Masters in **Toxicology**. The main subjects are ethics, animal welfare, alternative toxicity tests, quality assurance, toxicokinetics, organ toxicity, adverse outcome pathways, and computational toxicology. They usually cover a broad range of chemicals such as pesticides, cosmetics and environmental contaminants.
- The third category includes **Environment and Ecotoxicology** Masters, where subjects such as biostatistics, environmental economics, environmental biotechnology, ecotoxicology and immunology are taught. Their focus is on the behaviour and effects of toxicants in the environment.
- Some modules of Masters-level programmes are specifically about **Alternative Methods**. They typically cover strategies for replacing animals in basic and applied research and describe *in vitro* methods including cell culture and analytical chemistry, as well as internationally and regulatory accepted toxicological tests based on alternative methods.

Some lecturers responsible for the 94 Masters courses were interviewed to obtain additional insights into their programme and specific modules. These interviews covered 20 courses and comprised the questions listed in Box 4.

This analysis of Masters programmes confirmed the outcome of the previous JRC study i.e. Masters courses are available across the globe with most being offered in Europe and North America. Replacement is the most predominant Three Rs principle taught in the programmes and in the majority of cases there is emphasis on the importance of alternative methods. The demand to attend these Masters programmes is high and comes from students with different backgrounds. The qualification achieved

Box 4. Questions asked during the interviews of lecturers responsible for Masters courses

1. Describe the structure of the course and the emphasis given to each of the Three Rs;
2. Which teaching resources were used during the course and how did the lecturer keep current with Three Rs information and the state-of-the-art of alternative methods;
3. Which other resources would be useful to support the teaching of the Masters programme with respect to the Three Rs;
4. What was the level of demand for the course, and how did the career path of participants develop following the course.

will ultimately give access to a wide range of job opportunities in the area of non-animal experimental approaches.

According to those interviewed, **some educational resources are lacking**, namely textbooks, videos and resources providing more detailed information on new methods. In addition, a more active **collaboration between universities and research centres** running Masters programmes could attract more students and facilitate better knowledge sharing.

2.3 Interviews with experts in the Three Rs

Some interviews were also carried out with experts in the Three Rs in order to exploit their experience in the subject and their experience in the education sector. Experts supported **a focus on secondary school students**, raising the importance of starting Three Rs education as soon as possible. This would maximise long-term impact on practice and ensure the Three Rs principles become embedded in the attitudes and behaviour of students towards science, which form at an early age. For this reason, some thought that it would be desirable to start even earlier, such as in the last year of primary school with more general topics such as compassion, empathy and respect for animals and animal welfare, to stimulate ethical and critical thinking at an early age.

We found that the **awareness of existing tools and resources** among the experts was limited. The tools and resources very often have to be proactively searched for by educators and are introduced into a course on a voluntary basis. There was consensus on the fact that the available tools are designed for providing an introduction or general information rather than practical guidance or training in the Three Rs.

The key specialists recommended: **developing class plans** that can be readily implemented by teachers in the classroom with minimal or no adaptation; and investing in the continuous professional development of teachers and lecturers. This could facilitate widespread inclusion of Three Rs content on the curriculum through engaging the teachers themselves in the process.

A recommended option was to focus efforts on **raising awareness of the Three Rs among teachers in training** (i.e. those not yet in service) because this would have the highest impact in the long term and encourage culture change in secondary schools.

Some interviewees raised the importance of leaving some flexibility to adapt to local circumstances and the requirements of different disciplines (see Box 5), but also acknowledged that it would be possible, and indeed desirable, to introduce some degree of top-down/centralised input to encourage cooperation among different actors and monitor implementation.

Box 5. The Three Rs: an interdisciplinary subject

Although the tendency is to associate the Three Rs with science subjects, it does in fact lend itself to interdisciplinary and cross-topical subjects. Students can explore and integrate multiple perspectives from different angles based on their interests, involvement and background knowledge, offering greater opportunities to learn.

Indicative subject areas where the Three Rs could be covered include:

- Science: Biology (e.g. cell structure and function, molecular biology, pharmacology, toxicology, immunology, genetics); chemistry (e.g. analytical chemistry, biochemistry); engineering (bioimaging, cell and tissue engineering, biotechnology);
- Social Studies: Ethics; philosophy (e.g. debates about animal welfare, animal rights, etc.).
- History: History and civil society (e.g. how animal use in science developed, how legislation requiring animal testing emerged and what have been the shifts in public opinion; European Citizen's Initiative Stop Vivisection; current transition to non-animal methods in the EU).
- Law: European Union legislation; how Directives and Regulations are implemented with the examples of Directive 2010/63/EU and Regulation (EC) N° 1223/2009 (on cosmetics).
- Politics: Policy (e.g. the reasons why EU Directive 2010/63/EU was developed and its key elements).
- Economics: Business innovation (e.g. new start-ups and companies selling lab-produced human tissues such as skin[1]).

Interdisciplinary subjects are pivotal for encouraging connections between disciplines and helping students construct broad knowledge schema. Skills such as critical thinking, communication and analysis are important for this and are being developed throughout all stages of life.

Overall, these considerations strongly reinforce the concept that introducing the Three Rs into educational curricula may represent an exciting challenge for a better tailored education and training programmes for future generations.

1 *https://www.cnbc.com/2017/05/25/loreal-is-making-lab-produced-human-skin-to-curb-animal-testing.html*

3. How to do more

The European Centre for the Development of Vocational Training (Cedefop) has provided a definition of an education curriculum (Cedefop, 2010), however this term has diverse meanings across Europe.

The educational context of each Member State consists of several factors such as, curriculum development structures, policy priorities, human capacities, financial resources and educational traditions. Similarly, each curriculum is based on a national approach that accounts for top-down educational priorities set by each Member State.

3.1 Introducing the Three Rs in curricula

The implementation of the Three Rs into a curriculum should follow a combination of both top-down and bottom-up strategies conducted in a parallel and harmonised way to avoid overlaps or gaps and to make best use of resources (EURL ECVAM, 2017).

From the **top-down perspective**, it is important to understand how the Three Rs could fit into the education system in each Member State and to work with individual ministries of education and science to see how the Three Rs could be incorporated into school curricula at the next possible stage of curriculum development.

The International Bureau of Education of UNESCO has provided guidance on the process of developing and implementing curriculum frameworks (IBE-UNESCO, 2017). It proposes a flexible process that can be adapted to the situation and specific needs of each Member State that can be applied in an efficient and cost-effective way, within reasonable timeframes. It is

an approach that has been discussed between many stakeholders and supported by a number of experts (Fullan, 1994).

However, developing and implementing a curriculum framework can be a complex and slow process, requiring high-level support from the government, ministries of education and education planners. For this reason, a **bottom-up approach** can be considered complementary since it involves working with educators in schools and universities who can envisage ways to bring the Three Rs into their lessons through the use of different learning scenarios designed to suit a variety of different learning contexts.

Attempting to influence the curriculum in this way by targeting groups such as teachers, heads of schools or deans of university departments, who are usually not involved in the high-level process of decision-making, has been suggested and adopted in some cases (Nistor *et al.*, 2016). For example, many national and European[9] approaches exist that support the incorporation of STEM topics (Science, Technology, Engineering and Maths) in schools and universities. An example is climate change that has been integrated into existing curricula at several education levels. The One United Nations Climate Change Learning Partnership (UN CC:Learn, 2013) played a key role in this process by providing teaching materials for primary and secondary school educators.

9 *https://ec.europa.eu/social/main.jsp?catId=1223*

3.2 Learning scenarios: a key tool

Learning scenarios can be considered as a type of 'standard operating procedure' to guide teachers and lecturers in introducing a topic through a lesson, a set of lessons, a module or an entire course in their education programme. Based on a standard template, but remaining flexible and adaptable, learning scenarios offer ready-to-use education activities, resources, tools, background information on the topic and lesson instructions. They represent a valuable support tool to educators regardless of pedagogical approach, and their flexibility allows them to fit into various curriculum frameworks.

The granularity of learning scenarios may be different and several levels can be distinguished, from an activity within one or more lessons (lesson plan), to a module, a course, a summer school, or a skills training programme.

A module can be used as a building block, with multiple modules building into a course or programme. A summer school is an educational event that focuses on a particular theme at a time that is suitable for both university students and professionals to participate. A skills training programme is a series of educational tools to teach a particular skill or skillset. It can be implemented as a stand-alone programme (e.g. as continuing professional pevelopment) or as part of a broader education programme (e.g. Masters training, summer school).

Several education activities focusing on the Three Rs have already been developed, implemented and used in lessons, modules, summer schools and training courses by educators or pioneers of the Three Rs. In the sections below, some of these existing educational activities at both secondary school and university level have been contextualised in learning scenarios to be used by everyone in the education system to introduce the Three Rs as a subject in their curriculum.

The learning scenarios have been complemented with a set of **teaching resources**, which can be found on the education and training section of the JRC's EU Science Hub website[10]:

1. An online MOOC (with digital badge) to help teachers become knowledgeable on the Three Rs;
2. A series of infographics with links to more dynamic content;
3. A slide-set (Microsoft Powerpoint) that teachers can use flexibly (and can edit and re-purpose);
4. A storyboard to accompany the slides, providing further detail that teachers can use to build knowledge of the Three Rs.

10 *https://ec.europa.eu/jrc/en/eurl/ecvam/knowledge-sharing-3rs/education-and-training*

3.3 A selection of learning scenarios for secondary schools

Six learning scenarios have been developed by European Schoolnet[11] in partnership with the JRC through a co-creation process that involved 12 teachers and a validation step in schools prior to finalisation. Summaries of the learning scenarios are provided below and the full versions can be downloaded through the Scientix[12] platform he JRC data catalogue[13].

These six learning scenarios can be readily implemented by teachers or adapted according to their needs to introduce the Three Rs into their lessons and programmes.

11 *http://www.eun.org/*

12 *http://www.scientix.eu/pilots/pilot-3rs*

13 *https://data.jrc.ec.europa.eu/dataset/5803050b-bdc4-4032-bbda-f794a0fc58c0*

Learning scenario 1

Title: How society uses and misuses Animals

Topic-Subtopic: Animal welfare – Animals in society

Summary: In this learning scenario students are made aware of the different "uses and misuses" of animals in our societies, such as for the production of food and clothing, religion and cultural traditions, entertainment, pet-therapy, etc., and improve their own learning skills.

Learning outcomes:

- Identify the uses of animals in our society.
- Collect different opinions in students' schools.

Learning scenario 2

Title: To use or not to use live animals in science

Topic-Subtopic: Animal welfare – Animals in science

Summary: In this learning scenario pupils learn about the Three Rs principles. They are confronted with different methodologies used in science in order to raise awareness on the need or not of using live animals for scientific research. Through online and offline tools they will investigate the digestive, circulatory, reproductive and respiratory systems, while understanding the possibility and importance of research in science and medicine without using live animals. Biodiversity in aquatic and terrestrial ecosystems are also explored.

Learning outcomes:

- Understand why animals are used in science and what the ethical, societal and scientific challenges are.
- Raise awareness about science laboratory techniques without the use of live animals according to the Three Rs principles.
- Investigate the digestive, circulatory, reproductive and respiratory systems.

Learning scenario 3

Title: Sustainable science: the Three Rs

Topic-Subtopic: Sustainable science – the Three Rs

Summary: This learning scenario leads to the definition of the Three Rs principles and how these principles have been at the basis of the development of European legislation on the use of animals in experimental laboratories. Emphasis is given to the importance of the Three Rs in science for the development of alternative methods.

Learning outcomes:

- Learn about the rights of animals to be protected from their indiscriminate use in science.
- Discover how the Three Rs principles were introduced for the first time into European legislation and implemented at Member State level.
- Learn about the career profile of a practitioner who practices the Three Rs in their work.
- Examine how STEM topics including non-animal methods can support employment in the professions of the future.

Learning scenario 4

Title: Human-based science: where humans can do it on their own

Topic-Subtopic: Sustainable science – Human-based science

Summary: This learning scenario challenges the idea that animal testing is unavoidable due to the lack of alternative options to carry out research and conduct tests. Thus, the aim is to illustrate such innovative methods and the way they are being applied. Students will make use of creative thinking techniques to identify the positive aspects, the challenges and even think of potential innovative future applications. Students are provided with adequate information and will have to elaborate their findings and prepare an output, which will be used during a hypothetical funding-request interview. The output can take the form of a presentation, a model, a chart, a video or any other form the students would realistically be able to produce.

Learning outcomes:

- Introduce the different human-based methods that replaced animal methods.
- Make students aware of the application of human-based research in science.

Learning scenario 5

Title: Critical thinking: emotions versus facts

Topic-Subtopic: Critical thinking – Debate on acknowledging emotions and facts

Summary: This learning scenario aims at stimulating students to think critically about animal welfare in science. This critical thinking is introduced by organising a debate on animal welfare in the format of PlayDecide, Socratic seminar or debate. A public website has been created to be consulted by teachers and guide them in the preparation and organisation of the debate.

Learning outcomes:

- Illustrate the relation between science and society on an ethical and philosophical base.
- Illustrate the importance of scientific research in a healthy lifestyle.
- Create answers on sustainability problems concerning animal welfare.
- Students can think critically about emotions vs facts about animals used in science.
- Formulate well-built arguments in a critical debate.

Learning scenario 6

Title: Animal experimentation in scientific literacy

Topic-Subtopic: Critical thinking – science literacy

Summary: This learning scenario aims to understand and differentiate good science/bad science (fake news, pseudoscience, etc.), while pupils acquiring knowledge on animal experiments and animal welfare. By using critical thinking skills, pupils learn how to do a literature search about animal experimentation and then have a group discussion in the classroom. They comprehend how science works by carrying out a survey about the use of animals in scientific experiments in their own school and analysis the results as a final activity.

Learning outcomes:

- Improve pupils' critical thinking skills and promote science literacy based on the activities about animal testing.
- Learn how to formulate research questions relating to the use of animals.
- Collect data, draw simple graphs and interpret and communicate the results.

3.4 A selection of learning scenarios for universities and continuing education

Selected experts in the Three Rs working in higher education have provided a learning scenario on their own particular course with the aim of making them available to the target group who intends to use it - as it is or customised according to their needs - to introduce the Three Rs in their curriculum and course content. This collection of learning scenarios can also be seen as a collaborative activity towards further development and implementation.

The summaries of these learning scenarios are available below, while a full version of each can be downloaded through the JRC data catalogue[14].

14 *https://data.jrc.ec.europa.eu/dataset/5803050b-bdc4-4032-bbda-f794a0fc58c0*

Learning scenario 1

Alternative methods in toxicology

Owner: Master of Veterinary Biotechnology Sciences, University of Milan, Italy

Topics: Three Rs (Replace, Reduce, Refine), alternative methods in toxicology, stand-alone methods, integrated testing strategy, validation

Eligible student level: Bachelor degree

Teaching time: 30 hours - 3 hours per week

Summary: This module is divided into two parts. The theoretical part provides the description of methodologies and the Three Rs application in toxicological research, with particular interest aimed at new perspectives, thanks to the intervention of experts in the field. The practical part includes demonstrations with *in vitro* models for replacement, and examples of Integrated Testing Strategies. A specific lesson is reserved for the illustration of in silico models, provided in ad hoc facilities. A dictionary with an up-dated nomenclature is also provided to the students.

Learning outcomes:

- Learn about the Three Rs (Replace, Reduce, Refine): importance and vision
- Learn about existing alternative methods and applications in toxicology
- Understand the concept of stand-alone methods
- Learn *in vitro* and in silico approaches
- Comprise the key-steps of an Integrated Testing Strategy
- Importance of the development of new alternative methods
- Create a new mentality, and familiarise students with alternatives

Learning scenario 2

Toxicology and *in vitro* models

Owner: Master of Veterinary Biotechnology Sciences, University of Milan, Italy

Topics: *In vitro* models in toxicology, *in vitro* toxicity tests and applicability domain, practical approach in lab on the fundamental *in vitro* toxicity assays

Eligible student level: Bachelor degree

Teaching time: 30 hours - 3 hours per week

Summary: The purpose of the course is to provide tools and information on *in vitro* tests and models for toxicological studies, with a particular attention on emerging techniques. It is a mainly practical course and includes protocols on cytotoxicity assays, toxicity studies with *in vitro* epithelial barrier and permeability studies, *in vitro* models for endocrine disruptor activity, *in vitro* models for xenobiotics metabolism were provided to the students during the course. Students first became familiar with classical cytotoxicity assays i.e. cytokines release (IL-1alfa, TNF-alfa), LDH release, MTT test, NRU test and subsequently they learn the use of different *in vitro* models from 3D to epithelial barriers and their applicability in toxicology. *In vitro* study on metabolism and permeability studies are outlined. For each topic a specific protocol is provided and all the practical activity is set-up by mimicking the real test, so that students can actually work and learn from the practical point of view

Learning outcomes:

- Concept of the Three Rs, focus on Replacement and role of the *in vitro* approach;
- Knowledge of the principal *in vitro* tests/ assays;
- Information on the use of the *in vitro* methods in toxicology;
- Learn the applicability of different *in vitro* models in toxicology and the specific endpoints;
- Practical activity in the lab with specific *in vitro* protocols;
- Learn innovative methodologies, strategies and future perspective (i.e. spheroids, organoids).

Learning scenario 3

Safety Assessment of novel therapeutic agents: the functional observational battery (FOB)

Owner: University of Leeds, United Kingdom

Topics: Safety pharmacological assessments; animal physiology and behaviour; animal welfare; animal handling and restraint; experimental design; reproducibility and reliability of animal studies

Eligible student level: Undergraduate (Pharmacology, Physiology and related disciplines); experience of handling and restraint of the species (rat or mouse) used; prior knowledge and understanding of the use of the functional observation battery in the safety pharmacological assessment of putative new medicines

Teaching time: 3.5 hours

Summary: Functional observational battery (FOB) tests in laboratory animals are similar to clinical neurological examinations in humans in that they assess the presence and severity of behavioural and/or neurologic dysfunction. FOB tests are widely used in safety pharmacological studies because, if they are carried out by carefully trained observers, they can alert researchers to potential safety issues with novel pharmacological agents including sedative potential, motor impairments and abuse liability. If the FOB test highlights these effects, further, more specific safety pharmacological evaluations are carried out to confirm these findings. In this session, working in groups, students will gain hands-on practical experience of conducting an FOB on a rodent through animal handling, the assessment of rodent physiology and behaviour, and knowledge and understanding of how specific physiological or behavioural measures or indices can be modulated by different classes of pharmacological agents. Students will also have a greater appreciation of the impact of researcher expertise and skill on the reproducibility and reliability of data from animal studies

Learning outcomes:

By the end of this session, participants should have:

- A greater knowledge and understanding of rodent physiology and behaviour;
- A greater knowledge and understanding of the use of the FOB to assess the impact of pharmacological agents on rodent physiology and behaviour;
- Gained hands-on practical experience animal handling and of conducting functional observations in rodents;
- Gained experience of experimental design, team-working, leadership, problem solving; communication skills; reporting of scientific studies, and an increased ethical awareness.
- A greater appreciation of the care and skill required when undertaking physiological and behavioural studies in laboratory animals; and the impact of researcher skill and expertise on the reproducibility and reliability of data from animal studies.

Learning scenario 4

JRC summer school: non-animal approaches in science: challenges and future directions

Owner: European Commission's Joint Research Centre, Unit F.3 Chemicals Safety and Alternative Methods incorporating the EU Reference Laboratory for alternatives to animal testing (EURL ECVAM)

Topics: Legal obligations, strengths and limitations of animal and alternative methods, alternative (*in vitro* and in silico) methods and integrated approaches, future challenges.

Eligible student level: Postgraduate students and early-career scientists (max. 4 years after master/PhD)

Teaching time: 25h divided over 4 days. 13h are dedicated to networking activities

Summary: The aim of the JRC summer school is to share knowledge and experience on the latest non-animal approaches used in research and testing including *in vitro* methods and computational modelling and how those can be applied in regulatory safety assessment and biochemical research.

Learning outcomes:

- Examine the legal obligations and state of play of replacing animals for scientific purposes in the European Union and North America;
- Compare strength and limitations of animal vs alternative approaches;
- Discover novel applications of *in vitro* methods (e.g. developmental neurotoxicity testing, animal-free cultures, 3D cultures and organ-on-chip systems);
- Apply computational methods to specific cases (e.g. predictive toxicology and drug development, threshold of (eco-)toxicological concern for aquatic toxicity, biokinetic modelling, human in silico clinical trials;
- Combine non-animal methods into integrated solutions (e.g. biomarkers and mode of action in pharma development, high throughput methodologies, adverse outcome pathways, regulatory uptake of combined methods for skin sensitisation);
- Consider the future challenges of alternative methods (e.g. genomics data for chemical safety, alternatives to respiratory tract disease, endocrine disruptors, links between diseases and chemicals in the environment);
- Identify some of activities carried out in a lab where non-animal approaches are used;
- Defend an argument in a public debate and learning by preparing arguments (specific arguments were: A) are legal obligations necessary to support the Three Rs? B) Can computational methods provide stand-alone solutions? C) Do adverse outcome pathways have a future for regulatory toxicology? D) Can we do science without animal experiments?).

4. Conclusions

Knowledge sharing activities are crucial to achieving the aim of increased Three Rs uptake. In particular, knowledge-sharing through education and training activities is an effective and sustainable way to enlighten and inspire the next generation of scientists, instil an informed, open and creative mind-set at an early stage of educational life, and help to establish a belief and sense of purpose to make progress in life sciences through embracing of the Three Rs.

Through carefully assessing the state of play and bringing together Three Rs experts and educators, the JRC proposes a means to more effectively share Three Rs knowledge through education activities. This will in turn support improving animal welfare, reducing and ultimately replacing animals in science as well as in a regulatory testing context.

The JRC intends to continue efforts towards increasing the awareness and understanding of the Three Rs in the education arena. A next step is to embark on outreach activities engaging with education decision makers to seek ways of more systematically and comprehensively bringing the Three Rs into the classroom and lecture hall, working closely with education providers and responsible entities in the Member States.

Box 6. Recommendations

- Work should continue with educators, experts and education authorities to find the best way to support teaching the Three Rs at all levels, expanding understanding of our target audiences and their resource needs;
- More emphasis should be given to 'training the trainers';
- Consider widening the target for education actions to include the final year of primary school;
- Explore ways to raise awareness of careers related to the Three Rs, STEM-related or otherwise;
- Continue to provide practical guidance to education decision-makers and policy-makers on how to incorporate the Three Rs into the curriculum;
- Explore implementing Three Rs education in disciplines other than life sciences.

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