

The Rise of Three Rs Centres and Platforms in Europe*

Alternatives to Laboratory Animals

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Abstract

Public awareness and discussion about animal experiments and replacement methods has greatly increased in recent years. The term ‘the Three Rs’, which stands for the Replacement, Reduction and Refinement of animal experiments, is inseparably linked in this context. A common goal within the Three Rs scientific community is to develop predictive non-animal models and to better integrate all available data from *in vitro*, *in silico* and omics technologies into regulatory decision-making processes regarding, for example, the toxicity of chemicals, drugs or food ingredients. In addition, it is a general concern to implement (human) non-animal methods in basic research. Toward these efforts, there has been an ever-increasing number of Three Rs centres and platforms established over recent years — not only to develop novel methods, but also to disseminate knowledge and help to implement the Three Rs principles in policies and education. The adoption of *Directive 2010/63/EU* on the protection of animals used for scientific purposes gave a strong impetus to the creation of Three Rs initiatives, in the form of centres and platforms. As the first of a series of papers, this article gives an overview of the European Three Rs centres and platforms, and their historical development. The subsequent articles, to be published over the course of *ATLA*’s 50th Anniversary year, will summarise the current focus and tasks as well as the future and the plans of the Three Rs centres and platforms. The Three Rs centres and platforms are very important points of contact and play an immense role in their respective countries as ‘on the ground’ facilitators of *Directive 2010/63/EU*. They are also invaluable for the widespread dissemination of information and for promoting implementation of the Three Rs in general.

Keywords

3R, 3Rs, EU3Rnet, NAM, NAMs, new approach methodologies, novel approach methodologies, non-animal methods

Introduction

There have been a number of influential milestones in the development of the Three Rs field. After the exposure of major cases of animal abuse in post-war animal facilities, it became clear that improvements and standardisation in animal husbandry and hygiene were necessary, and also that the animal welfare aspects of experimental procedures should be given much closer attention. This was especially in light of the fact that a much greater future need for laboratory animal use was envisioned. The development of

the Three Rs field was very closely linked to the use of animal experiments in toxicology and drug development, and scandals (e.g. Elixir Sulfanilamide and thalidomide) raised awareness of the need for more thorough and rationale-based preclinical research. In addition to the further development and refinement of animal experiments, questions on how these experiments could be carried out ethically were also raised. This led to work commissioned by UFAW (the Universities Federation for Animal Welfare) in the UK, in which close co-operation between laboratory animal scientists, laboratory managers, animal breeders and

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The *Appendix* lists the acronyms and abbreviations used throughout the article.

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scientists resulted in the so-called ‘L.A.B. Surveys’ of 1952 onwards. The ground-breaking book by William Russell and Rex Burch, *The Principles of Humane Experimental Technique*¹ (http://altweb.jhsph.edu/pubs/books/humane_exp/het-toc), was published in 1959 and was based on this UFAW initiative. The Three Rs concept was first described in this book, and as such it is now considered to be the first standard work in the field. Other milestones in the Three Rs field include:

- the 1964 Declaration of Helsinki of the World Medical Association on Research Involving Human Subjects (“Biomedical research involving human subjects must conform to generally accepted scientific principles; it should be based on adequate laboratory and animal experimentation and a comprehensive knowledge of the scientific literature.”);²
- a symposium organised by UFAW in 1969 and the resulting book *The Use of Animals in Toxicological Studies*;^{3,4}
- the founding of FRAME (the Fund for the Replacement of Animals in Medical Experiments) in the UK, by Dorothy Hegarty in 1969; and
- the publication, in 1978, of D.H. Smyth’s *Alternatives to Animal Experiments*,⁵ which has many practical and constructive suggestions.

Later key developments were the foundation of ZEBET (Zentralstelle zur Erfassung und Bewertung von Ersatz- und Ergänzungsmethoden zum Tierversuch) in Germany in 1989, as the first governmental institution with the mandate to reduce animal experiments on a scientific basis, and the foundation of ECVAM (European Centre for the Validation of Alternative Methods) at the EU Research Centre in Ispra, Italy (<https://ec.europa.eu/jrc/en/eurl/ecvam>) in 1993. In the international context, the foundation of Johns Hopkins Center for Alternatives to Animal Testing (CAAT; <https://caat.jhsph.edu>) in 1981, and ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods) by the US federal authorities in 1997, were also important steps.

Many laboratory animal facilities worldwide contributed to the development of the Three Rs from the 1960s onwards, without necessarily calling themselves ‘Three Rs centres’. Increasing violence against animal research in the 1990s stimulated the formation of ecopa (European Consensus-Platform for Alternatives). This was formally agreed during the *Third World Congress on Alternatives and Animal Use in the Life Sciences*, in Bologna, Italy, in 1999. Members of ecopa were, and still are, national consensus platforms that meet the criterion of representation of all four main stakeholder parties (government, industry, academia and animal welfare organisations) in their governing body. Moving forward

into the new era of the 21st century, notable relevant milestones in EU legislation included:

- in 2002, the 7th Amendment of the EU Cosmetics Directive 76/768/EEC,⁶ which introduced marketing bans for cosmetic products and their ingredients when tested on animals, and was later converted into Regulation (EC) No. 1223/2009 on cosmetics products;⁷
- the EU legislation on REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) (EC 1907/2006);⁸
- Directive 2010/63/EU on the protection of animals used for scientific purposes;⁹ and
- the communication from the European Commission to the European Parliament and the Council on the animal testing and marketing ban and on the state of play in relation to alternative methods in the field of cosmetics with a final deadline of 11 March 2013,¹⁰ which was based on the EU Cosmetics Regulation (EC) 1223/2009.¹¹

Under REACH, companies bear the burden of proof regarding the safety of their products and, to comply with this regulation, they must identify and manage the risks associated with the substances that they manufacture and place on the market in the EU. They must demonstrate to the European Chemicals Agency (ECHA) how the substance can be used safely and must provide users with information on risk management measures. This led to tension between the legal requirement for animal experiments to test the toxicological properties of chemicals and the demands of upholding the Three Rs principles. The EU Directive 2010/63 was the first time that the Three Rs were specifically mentioned in EU legislation (although the Council of Europe Convention ETS 123 of 1986, which came into effect on 1 January 1991, mentions the same principles, but not so explicitly), and thus many European countries began to implement the Three Rs in their national legislation.¹² While REACH states in Article 1 “The purpose of this Regulation is to ensure a high level of protection of human health and the environment, including the promotion of alternative methods for assessment of hazards of substances...”, in effect, the legislation represents the most extensive animal testing programme of all time.¹³ In contrast to REACH, the ban on cosmetics in 2013 was hailed as a major success in the Three Rs field, though this was put into question very recently.¹⁴

With the publication and implementation of EU Directive 2010/63, there was an increase in the establishment of national and international Three Rs initiatives, which often led to the formation of Three Rs centres and platforms. Many countries did not have national consensus platforms that satisfied ecopa’s requirements of quadripartite

Table 1. A list of participating Three Rs institutions.

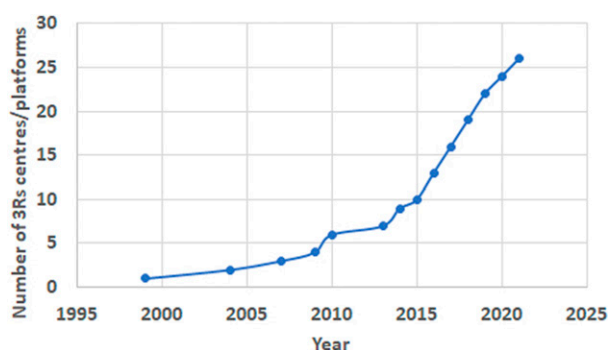
Name of the institution	Country	Type of institution	Website address
RepRefRed Society	Austria	Society	https://www.reprefred.eu/EN
Innovation Centre–3Rs (IC-3Rs)	Belgium	Centre	https://www.ic-3rs.org/
3Rs Centre Czech Republic	Czech Republic	Centre	https://www.szu.cz/
The Danish 3R-Center	Denmark	Centre	https://en.3rcenter.dk/
BB3R–Freie Universität Berlin	Germany	Platform	https://www.bb3r.de/
CAAT Europe	Germany	Centre	https://www.biologie.uni-konstanz.de/leist/caat-europe/
Charité 3 ^R	Germany	Centre	https://charite3r.charite.de/en/
ICAR3R–3R Centre JLU Giessen	Germany	Centre	https://www.uni-giessen.de/fbz/zentren/icar3r
Leibniz Alternatives at IUF Research Institute for Environmental Medicine	Germany	Platform	https://en.leibniz-alternatives.de/
R2N	Germany	Three Rs network	https://r2n.eu/home-2/
TARCforce3R	Germany	Centre	https://www.unimedizin-mainz.de/tarc-force-3r/
3R Centre Rhine Neckar	Germany	Centre	https://en.3r-rn.de/
VZET Hannover	Germany	Three Rs network	https://www.tiho-hannover.de/vzet
Trinity College London	Ireland	Centre	https://www.tcd.ie/comparativemedicine/
Centro 3R	Italy	Inter-university platform	https://www.centro3r.it/
Luxembourg 3Rs Platform	Luxembourg	Platform	https://www.en.uni.lu/ https://www.list.lu/
Ncad	Netherlands	National committee	https://english.ncadierproevenbeleid.nl/
3Rs-Centre of the Utrecht University and University Medical Centre Utrecht	Netherlands	Centre	https://www.uu.nl/en/organisation/3rs-centre/
Norecopa	Norway	Platform	https://norecopa.no/
National Centre for Alternative Methods to Toxicity Assessment (CMA)	Poland	Centre	https://twinalt.com/partners-lodz/
i3S	Portugal	Centre	https://www.i3s.up.pt/
ROCAM	Romania	Centre	http://rocam.usamvcluj.ro/
Slovak National Platform for 3Rs (SNP3Rs)	Slovak Republic	Platform	https://www.snp3rs.com/
CMCIB-IGTP–Comparative Medicine and Bioimage Centre of Catalonia, Germans Trias i Pujol Research Institute	Spain	Centre	https://www.cmcib.cat/
Swedish 3Rs Center	Sweden	Centre	https://jordbruksverket.se/languages/english/the-swedish-3rs-center
3RCC	Switzerland	Centre	https://swiss3rcc.org/
National Centre for the 3Rs (NC3Rs)	UK	Centre	https://www.nc3rs.org.uk/
The Ukrainian 3Rs Centre	Ukraine	Centre	https://nuft.edu.ua/en/nnixt/ktzpkp/

representation in their governing body, even though they did have active Three Rs centres. Thus, alternative attempts were made to network the Three Rs centres and platforms at the European level. Important networking events were organised by the initiative of EURL ECVAM in 2015 and 2016.¹⁵

As a result of the formation of an ever-increasing number of Three Rs centres and platforms, numerous networking meetings were organised after the ones in 2015 and 2016. The next one was held during the EUSAAT conference in Linz, Austria, in autumn 2018 (hosted by Prof. Monika Schäfer-Korting, of Freie Universität Berlin (FU Berlin)),

Table 2. An index of the countries featured in this article.

Country	Institution	Page
Austria	RepRefRed Society	95
Belgium	IC-3Rs	96
Czech Republic	3Rs Centre Czech Republic	97
Denmark	Danish 3R-Center	98
Germany	BB3R CAAT-Europe Charité 3 ^R ICAR3R (Interdisciplinary Centre for 3Rs in Animal Research) Leibniz Alternatives R2N TARCFORCE 3R 3R-Centre Rhine-Neckar VZET	98
Ireland	Trinity College Dublin	104
Italy	Centro 3R	105
Luxembourg	Luxembourg 3Rs Platform	106
Netherlands	NCad 3Rs-Centre of the Utrecht University and University Medical Centre Utrecht	106
Norway	Norecopa	107
Poland	CMA	108
Portugal	i3S	109
Romania	ROCAM	110
Slovak Republic	SNP3R	111
Spain	CMCiB	113
Sweden	Swedish 3Rs Center	113
Switzerland	3RCC	114
United Kingdom	NC3Rs	114
Ukraine	Ukrainian 3Rs Centre	115

**Figure 1.** The cumulative increase in the number of Three Rs centres and platforms in Europe over recent years.

and another in June 2019 at the FELASA conference, where, in addition to more than 20 Three Rs centres and platforms, there was again an important exchange of information with representatives from EURL ECVAM. From these discussions, five important areas for further development of Three Rs initiatives and topics were identified, namely: Dissemination, Education, Implementation,

Scientific Quality/Translatibility and Ethics, and corresponding working groups were set up to cover each of these five areas. In addition, the name of the network was defined and was called 'EU3Rnet' (European Network of 3R Centres). A consensus statement from EU3Rnet, signed by 25 members, was published recently¹⁶ and many joint activities, such as the co-authorship of articles and grant proposals, are in progress.

For this series of *ATLA* articles, 26 Three Rs institutions contributed information — introducing themselves, describing their history, detailing their financial and organisational structure, and highlighting some of their key initiative-leading members, past and present. They have provided insights into their current work, Three Rs priorities and their plans for the future. In view of the wide diversity of such organisations, each centre was allowed to contribute to this overview in its own way, with minimal editorial control exerted over the length and content of the individual contributions. These various centres and platforms can serve as useful role models to other countries, by giving examples of how to organise and establish a Three Rs centre or platform, as well as offering guidance on dealing with a

range of impediments that might be encountered. The participating Three Rs institutions, including their websites for contact details, are listed in Table 1.

Since the Three Rs field and the establishment of Three Rs centres and platforms is very dynamic, we do not claim to present here an exhaustive overview, rather we have attempted to provide a representative snapshot of the current situation. For example, a French Three Rs centre was expected to be inaugurated in 2021 and will hopefully join EU3Rnet soon after its establishment. However, this new centre has not been considered in this article. With regard to the newly-formed EU3Rnet, Norecopa has produced an interactive map of European centres and other global Three Rs networks,^{17,18} with links to the descriptions of each centre, which could also be a valuable resource for interested readers. As the reader will recognise, the diversity of Three Rs centres and platforms is extensive, and there is no real standard definition of a ‘Three Rs centre’ — for example, over 30 relevant groups currently participate in EU3Rnet.

This first article of the series looks at the different history of the Three Rs in the individual countries (as indexed in Table 2) and focuses on specific centres and platforms. This is important to better understand the circumstances of how and why certain Three Rs centres and platforms developed. Clear evidence of the rapid rise in Three Rs centres and platforms in Europe can be found by looking at the cumulative numbers established over time, as shown in Figure 1. Looking at the founding dates of the 26 institutions participating in this article, it is evident that there was a significant increase in the number of centres and platforms after the adoption of *Directive 2010/63/EU* and its transposition into national law (on 1 January 2013). In fact, it was precisely in 2013 that the rapid increase in the number of Three Rs centres and platforms began in Europe generally.

At this point, we would like to refer to an excellent book in which the reader can find a wealth of detail about the development of the Three Rs field: *The History of Alternative Test Methods in Toxicology*, was collated by Michael Balls, Robert Combes and Andrew Worth as editors, in 2018.¹⁹ This current article does not claim to go into the historical background of individual countries and regions in as much detail as the book, but rather aims to provide a brief outline and highlight the specific characteristics and milestones of their Three Rs centres and platforms.

Historical development of Three Rs centres and platforms in Europe

Austria

The Aktiver Arbeitskreis gegen Tierversuche (Working Group Against Animal Experiments) was founded in 1985. After lengthy discussions, the Arbeitskreis für die Förderung von tierversuchsfreier Forschung (AFTF)

(Working Group for the Promotion of Animal-Free Research) developed from the original group in 1988. The AFTF pursued the concept of the Three Rs and was no longer, like its predecessor, an anti-animal experimentation association. After the AFTF (Working Group for the Promotion of Animal-Free Research) had reached its limits, the Zentrum für Ersatz- und Ergänzungsmethoden zu Tierversuchen (ZET) (Centre for Replacement and Complementary Methods to Animal Experiments) was founded in 1996. This new centre, with a more professional structure, took over the previous tasks of the AFTF on the one hand and, on the other hand, expanded its area of responsibility to include its own research activities. ZET was meant to be a national reference centre for Austria.²⁰ At the same time, in the late 1980s and early 1990s, stronger efforts to develop the field of alternative methods in middle Europe resulted in the foundation of MEGAT (Middle European Society for Alternatives to Animal Experiments) in 1993, with members from Germany, Switzerland and Austria. MEGAT aimed to promote dialogue between animal welfare organisations, academia, industry and the government and/or regulators. In 1994, MEGAT organised the first congress on the Three Rs in Linz, Austria. During these times, commitment in Linz from individuals such as Harald Schöffl and the Schöffl family, as well as from animal welfare groups, politicians and the State Government of Upper Austria, in conjunction with the driving forces of key people like Horst Spielmann, Wolfgang Tritthart (from Graz), Franz Gruber, Walter Pfaller, Harald Appl, and ZET itself, were indispensable for the success of the Three Rs conferences held in Linz. In addition, the important role of Dr Wolf Frühauf (Department Head of the Ministry for Research and Education) must be greatly acknowledged, as he was responsible for the implementation of the Austrian animal welfare legislation and the EU *Directive 86/609*.

For activities relevant to the development of the Three Rs field and MEGAT, Fonds für versuchstierfreie Forschung (FFvFF), ALTEX, the 3R-Foundation with Interpharma (a drug company based in Basel), and the Döerenkamp-Zbinden Foundation with Franz Gruber, were all key players from Switzerland. In Germany, major roles were played by ZEBET at the BGA, BgVV and BfR in Berlin (see the *Germany* section for full details), the foundation of SET (in 1986), the Academy for Animal Protection (Akademie für Tierschutz), the University of Konstanz and Thomas Hartung. In subsequent years, colleagues from other countries such as the Netherlands or the Czech Republic joined MEGAT, which led to the idea of renaming it as the European Society for Alternatives to Animal Testing (EUSAAT). This process was finalised in 2008 and EUSAAT has been active in the field since then.

EUSAAT actively promotes the protection of experimental animals according to the Three Rs principles proposed by William Russell and Rex Burch in 1959, in their

ground-breaking book *The Principles of Humane Experimental Technique*.¹ It serves as a European Three Rs platform, offering expertise and networking opportunities (at national, European and international level), and supporting educational programmes in close co-operation with NGOs, regulators, research institutions and industry to improve and implement humane experimental techniques, as well as advanced *in silico* and *in vitro* methods. EUSAAT focuses its activities on all areas of the life sciences that rely on the use of experimental animals, including pharmaceutical research, cosmetics industry, chemicals industry, regulatory testing, as well as basic science. To achieve these goals, EUSAAT hosts the European Three Rs congress at the University of Linz (Austria), which initially, in 1991, was organised by local animal welfare groups, and then was organised under the patronage of MEGAT and, since 2008, of EUSAAT. Currently, there are three Three Rs organisations active in Austria: the RepRefRed Society, together with the MUI animalFree Research Cluster in Innsbruck, and EUSAAT.

The RepRefRed Society: The RepRefRed Society (Gesellschaft zur Förderung von Alternativen Biomodellen) was founded on 18 January 2016 by the researchers Birgit Reiningner-Gutmann and Beate Rinner from the Medical University of Graz. With the support of Doris Wilflingseder from the Medical University of Innsbruck and Roberto Plasenzotti from the Medical University of Vienna, the society aims to promote implementation of the Three Rs. In order to achieve this, the association focuses on the active exchange of information among researchers through topical meetings and events. The increased transparency of protocols should lead to *refinement* (improvement of animal welfare), as well as *reduction* (reduction in the number of animals used in experiments). The network should also help to strengthen the *replacement* of animal experiments in scientific research. The RepRefRed Society is currently promoting the Three Rs, on a national and international basis, by connecting with other like-minded centres or organisations. Additionally, the society is currently building the Austrian 3Rs Center, which should function as a mainly national contact for the research community and for the public on issues surrounding the Three Rs.

Belgium

The Belgian situation is challenging, as there are three regions (Flanders, Brussels and Wallonia) which are governed by different political parties. Significant efforts have been made to create a Belgian National 3Rs Centre, but these have failed because of differences in regional administration procedures. In most of the universities in Belgium, the topic of the Three Rs is covered, but in a non-uniform manner that lacks a joined-up approach. Thus,

currently there is only one Three Rs centre — the Innovation Centre 3Rs (IC-3Rs), located at the Vrije Universiteit Brussel (VUB) — which is trying to unite all Three Rs efforts in Belgium. Activities associated with the development and promotion of alternative methods and Three Rs platforms date back to the late 1980s and early 1990s. At that time, under the auspices of HRH Prince Laurent of Belgium (of the Group for the Reflection on the Quality of Life and the Environment), attempts were made to promote the Three Rs. This challenge was later taken on by Prof. Vera Rogiers at the VUB and Prof. Marcel Roberfroid at the Université Catholique de Louvain. In the mid-1980s, the European Research Group for Alternatives in Toxicity Testing (ERGATT) was founded, and Prince Laurent became its president in 1997. Prince Laurent had already taken over the patronage of a platform with the aim of validating and promoting alternative methods, which finally led in 1999 to the foundation of the Belgian Platform for Alternative Methods (BPAM), whose presidency Prince Laurent also accepted. BPAM was managed by Vera Rogiers and Sonja Beken, and brought together representatives from government, academia, industry and animal welfare; it was primarily concerned with the dissemination of the Three Rs through the publication of newsletters and books. Relatively soon after its creation, BPAM became part of the Prince Laurent Foundation and was actively involved in organising meetings and raising awareness of the importance of introducing novel *in vitro* and *in silico* technologies into science, regulatory audits and training. At the Third World Congress on Alternatives and Animal Use in the Life Sciences in Bologna in 1999, Vera Rogiers and the BPAM played a decisive role in the founding of ecopa, whose membership structure was similar to that of BPAM. The national platforms aiming to be members of ecopa had to include members from government, academia, industry and animal welfare organisations, in order to cover all relevant stakeholders in the field. Vera Rogiers was the chair-person of ecopa for 10 years and worked closely together with José Castell (Co-chair) of the University of Valencia (Spain) and Bernward Garthoff (Secretary) of Bayer/Crop Protection (Germany) during this period. Member platforms from several countries (Norway, Sweden, Hungary and Poland) joined ecopa under the Belgian umbrella. Ecopa organised two symposia per year and supported several European research projects.

IC-3Rs: With the implementation of *Directive 2010/63/EU* in the national legislation of Belgium, the three regions (Flanders, Brussels and Wallonia) each became responsible for implementation of the Three Rs (6th state reform, 1 July 2014). To fulfil this mandate in Flanders, the project Re-Place was initiated, coordinated by Birgit Mertens (Sciensano) and Vera Rogiers (VUB). The Brussels-Capital region has joined the project, but the Walloon region has

not done so as yet, although significant efforts have been undertaken to facilitate its inclusion. The Re-Place project had a special focus on New Approach Methodologies (NAMs), aiming to highlight and provide information on the existing expertise on NAMs in Belgium. In parallel with these developments, the Innovation Centre 3Rs (IC-3Rs) was launched on 25 September 2017 at the Brussels Health Campus, as part of the *In Vitro* Toxicology and Dermato-Cosmetology (IVTD) research group, chaired by Vera Rogiers. The IC-3Rs aims to be active specifically at the regional level, in terms of the dissemination, education and promotion of Three Rs alternative methods that are applicable to human-specific cutting-edge research.

An integral part of the dissemination activities of the IC-3Rs is the organisation of their yearly Symposium on Alternative Methods. A notable highlight was that, in conjunction with the Symposium organisation in 2017/2018, Maurice Whelan (current head of EURL ECVAM) and Vera Rogiers both received the Francqui Chair in Belgium, at the VUB and the University of Antwerp, respectively. In this regard, they both gave a series of lectures covering the development and active use of alternative methods in toxicology, and in basic and applied research. Recently, philanthropist Mireille Aerens bequeathed her assets to the VUB, to support the IC-3Rs and IVTD. This will enable the IC-3Rs to work sustainably and expand its research and its website, as well as further develop its organisational structure by establishing a Steering Committee.

Czech Republic

The concept of the Three Rs was introduced in the Czech Republic after the Velvet Revolution in 1989, when contact among scientists involved in animal research and their counterparts around the world intensified. Collaborations were established with renowned scientists working on the development of alternative methods, namely: Horst Spielmann (ZEBET, Germany), Michael Balls (FRAME, UK), Thomas Hartung (JRC-EC, ECVAM, Italy) and Björn Ekwall (Scandinavian Society for Cell Toxicology, Sweden). In 2001, an international symposium on the promotion of the Three Rs concept in Slovakia, Slovenia and the Czech Republic took place in Prague. The meeting was organised by the Charles University Faculty of Medicine in Hradec Králové, the National Institute of Public Health (Prague) and the Central Commission for Animal Welfare (Prague), as a result of an ECVAM initiative to promote the Three Rs in countries of Central and Eastern Europe. Motivated by the proposal for the creation of national consensus platforms for alternatives, which was presented at the 3rd World Congress on Alternatives in Bologna (1999), a Three Rs Czech Consensus Platform for Alternatives (Czecopa) was founded in 2001. It was registered in the Czech Republic and established as a member of ecopa in 2003 by Dr Dagmar

Jírová of the National Institute of Public Health in Prague. The platform comprised stakeholders from all four parties (NGO, academia, government and industry) on the basis of consensus and became a communication centre for the Czech Republic, ensuring scientific co-operation, education and the broad dissemination of public information.

Members of Czecopa presented potential replacements for animal tests at various national and international conferences and workshops, focusing on the progress, development and legal acceptance of alternative methods in the early 2000s, especially in the area of cosmetic products and their ingredients following the 7th amendment to the Cosmetics Directive in 2003 and *Regulation (EC) No. 1223/2009* on cosmetic products. The dissemination of information was enhanced by the participation of Czech experts in the ECVAM Scientific Advisory Committee (ESAC), and close co-operation between the Central Commission for Animal Welfare at the Ministry of Agriculture, the Czech Laboratory Animal Science Association CLASA (a member of FELASA), researchers, veterinarians, technicians and other experts from the field of animal experimentation. Czecopa members and associated scientists have since been closely involved with ECVAM, EUSAAT, ESTIV, SCCT and the OECD as experts or co-organisers of workshops and conferences. Following the adoption of *Directive 2010/63/EU* on the protection of animals used for scientific purposes, which was implemented into Czech national law (*Act 246/1992 Coll.* as an amendment in 2012), the Three Rs became an integral part of animal science in the Czech Republic. Since 2012, each project involving the use of experimental animals has been evaluated by independent authorities, which do not approve any projects that do not take into account all the Three Rs principles. The evaluation focuses not only on the replacement of animal methods with *in vitro* procedures, but also on the use of any new scientific experimental techniques, connected with reduction or refinement, as they emerge. The international contacts made by Miroslav Cervinka (Charles University Faculty of Medicine in Hradec Králové) and Dagmar Jírová (National Institute of Public Health in Prague) in the 1980s were crucial to the recognition of the Three Rs in Czechoslovakia. As a result of the increasing involvement of Czech scientists in the development and application of alternative methods, the 9th World Congress on Alternatives and Animal Use in the Life Sciences took place in Prague, in August 2014. Czecopa members were nominated to join the organising committee and the Congress Co-chairs were Dagmar Jírová and Horst Spielmann.

3Rs Centre Czech Republic: This centre was created in 2019 at the National Institute of Public Health in Prague, from the original Three Rs Czech Consensus Platform for Alternatives (Czecopa), following a meeting of Three Rs platforms and centres at the EUSAAT Conference on Alternatives

2018 in Linz, Austria. The National Institute of Public Health in Prague was appointed by the Ministry of Agriculture as a contact point to provide advice on the regulatory relevance and suitability of alternative approaches proposed for validation (i.e. PARERE), and the NRL for Experimental Immunotoxicology was nominated and approved as a qualified laboratory for validation studies, and thus joined the European Union Network of Laboratories for the Validation of Alternative Methods (EU NETVAL) at EURL ECVAM. Members of the 3Rs Centre and CLASA (Czech Laboratory Animal Science Association) were involved in the scientific and organising committees of the 14th FELASA Congress, which was held in Prague in June 2019.

Denmark

Denmark is strong in pharma and, consequently, experimental animals have been used routinely for preclinical studies. *Replacement* has not been prominent, and it was initially considered to be mostly relevant in universities; however, it was later taken up by the pharmaceutical industry and gained ground in research, education and testing. The Danish 3R-Center has granted projects including, and focusing on, *replacement*, which must be considered a step forward.

Danish 3R-Center: As early as 2005, the Danish government decided to establish the Danish Consensus Platform for Alternatives to Animal Experiments (DACOPA), under the European network ecopa (European Consensus-Platform for Alternatives). The purpose of DACOPA was to bring together representatives of animal protection organisations, private-public research and authorities, to seek consensus on animal testing issues with a particular view to promoting the Three Rs. DACOPA consisted of a chairperson and two representatives of each of the four groups (governmental, science, industry and animal welfare), which provided an opportunity to share lessons learnt and discuss how to promote the Three Rs in Denmark and abroad in the best possible way. However, DACOPA was challenged by a lack of funding for launching research projects and secretarial assistance to help them to perform the various tasks that they wished to undertake. This was not satisfactory for the groups of stakeholders, which was expressed in a stakeholder analysis carried out in 2011–2012 among all interested parties in the field of laboratory animals. Almost unanimously, the feedback was that Denmark should either set-up a Three Rs centre inspired by the British NC3Rs and the German ZEBET, or allocate sufficient funding to DACOPA. Following negotiations between the Ministry of Food, the pharmaceutical industry and a number of animal welfare organisations, in the spring of 2013 it was agreed to establish the Danish 3R-Center with a scientific board, its own budget, research project funding and a secretariat.

Germany

The world's first governmental centre for alternative methods was ZEBET (Zentralstelle zur Erfassung und Bewertung von Ersatz- und Ergänzungsmethoden zum Tierversuch; in English: Central Office for the Registration and Evaluation of Alternative and Complementary Methods to Animal Experiments) at the German Federal Institute for Risk Assessment (BfR) in Berlin. It was founded in 1989, led by Horst Spielmann until 2007, and commissioned by the Federal Government to limit the use of animals for scientific purposes to the indispensable minimum and to develop alternatives to animal experiments. Since 1994, it has been advised in this task by a commission consisting of representatives from science and industry, animal welfare organisations and national state authorities. ZEBET developed alternative methods, promoted research and advised authorities on questions of scientific animal welfare on a national and international level. At this time, the European Pharmacopoeia also specified a gradual withdrawal away from the use of animals for regulatory testing purposes. Thus, it became permissible to conduct drug testing by using Three Rs alternative methods instead of using the animal tests mentioned in the pharmacopoeia, as long as it could be demonstrated beyond doubt that the quality specifications for the product were met. For instance, vaccine testing in animals should generally be carried out with as few animals as possible, avoiding pain and suffering. As early as 1997, it was pointed out that the pharmacopoeia regulations for vaccines and sera offered many starting points for introducing improvements in terms of the Three Rs. In the meantime, numerous animal experiments have been replaced by alternative methods, or improved according to the Three Rs principles. The Paul Ehrlich Institute has been working continuously on the development and implementation of Three Rs alternative methods for the quality control of biomedical medicinal products.²¹ Another important influence at the time was from the field of laboratory animal science — for example, the ideas of Prof. Arnold Spiegel and Prof. Klaus Gärtner.²² Activities focused on developing knowledge and expertise, as well as enhancing standardisation (animals, facilities) and implementing regulation. The establishment of centralised animal facilities was a step toward better care for the animals. This all reflected an increased responsibility toward the Three Rs. Within the last 10–20 years, the field has become more diverse. Groups with no history in animal research (e.g. ELSI researchers; ELSI = ethical, legal and social implications), as well as groups with extensive previous biomedical activities, have become active in the Three Rs field. This opened the field considerably. In 2010, the Center for Alternatives to Animal Testing in Europe (CAAT-EU), located at the University of Konstanz, was founded.²³ CAAT-EU coordinates the transatlantic activities

to promote the development of new and improved methods in toxicology together with CAAT at Johns Hopkins University, Baltimore, USA.

Initiated through EU *Directive 2010/63/EU* on the protection of animals used for scientific purposes, the Three Rs principles have found their way into the German legislation with the amendments of the *Animal Protection Act* and the *Animal Protection Act for Test Animals* in 2013. Since then, every scientist conducting animal experiments and applying for approval from the competent authorities must adhere to the Three Rs and answer the following questions as part of the approval process:

- Are there alternative methods or strategies for the research question to avoid the use of animals?
- Is the number of animals that are planned to use reduced to an absolute minimum? and
- Is suffering of animals kept to the very lowest possible level?

This legislative activity led to the opening of the German Centre for the Protection of Laboratory Animals (Bf3R), at the German Federal Institute for Risk Assessment (BfR), in 2015. Also in 2015, ZEBET was incorporated into the Bf3R.

From both a national and an international perspective, Berlin has an unusually rich research landscape in the field of the Three Rs. Three Berlin-based institutions have set themselves the goal of undertaking Three Rs research and training.

- First, the BfR performs the role of the main German centre for the protection of laboratory animals via the work of the Bf3R, and coordinates all associated activities nationwide.
- Second, an important starting point was the acquisition of the BMBF-funded Berlin-Brandenburg Research Platform (BB3R), under the leadership of FU Berlin. The current spokesperson is Prof. Burkhard Kleuser, who succeeded Prof. Monika Schäfer-Korting in this role in 2020. Several partner institutions collaborate within the BB3R, to strengthen undergraduate and postgraduate education in the area and to perform Three Rs research. Furthermore, FU Berlin is home to the Institute for Animal Protection, Animal Behaviour and Laboratory Animal Science.
- Third, Charité–Universitätsmedizin Berlin established Charité 3^R, which is a faculty-overarching structure to foster the implementation of the Three Rs in the institution.

The establishment of BB3R in 2014 was a milestone, not only for the Three Rs in Berlin, but also for Germany as a whole. This is where Berlin-based systematic research in

this area began and where excellent young scientists are trained to a high level in alternative methods and animal-friendly working techniques at the integrated BB3R graduate college. This development is partly due to years of earlier work by a group of pharmacologists at the FU Berlin, headed by Prof. Monika Schäfer-Korting. This group assumed responsibility for issues surrounding *reduction* and *replacement* in several BMBF and EU-funded consortia, including industrial partners, for around 20 years. The development and validation of alternative test methods for the safety testing of chemicals in contact with human skin, and subsequent collaboration with industry and ZEBET, enabled the validation of many *in vitro* methods, e.g. for testing skin penetration and genotoxicity.

In 2018, Charité 3^R was founded at the Charité–Universitätsmedizin Berlin, with the overarching goal of improving biomedical research in order to accelerate translation toward improved patient therapy and care.²⁴ In addition to its activities in Three Rs education, Charité 3^R specifically pursues efforts aimed at engaging the scientific community within the Charité–Universitätsmedizin Berlin and Berlin as a whole, as well as reaching out to policy-makers and the general public.

Clearly highlighting the importance of the Three Rs concept in laboratory animal science in Germany, a number of additional Three Rs initiatives were established throughout Germany in this same time-frame. These include:

- ‘Replace and Reduce from Lower Saxony’ (R2N), which was created in Hannover in 2017, with the support of the Lower Saxony Ministry of Science and Culture (MWK), to promote the development of alternative methods and thus to replace as many *in vivo* experiments as possible and so reduce the numbers of laboratory animals used.
- ICAR3R (Interdisciplinary Centre for 3Rs in Animal Research) at the Justus Liebig University, in Giessen, which was established in 2018 to address replacement, refinement and improvement of the Three Rs. The ICAR3R not only develops its own new approaches, but also advises and promotes networks between the different scientific disciplines involved in research within the Central Hessen area, in order to expedite its goals.

In the context of the importance of the Three Rs in Germany, a very broad picture has emerged of the numerous institutes, centres, platforms, scientific working groups and individuals that have been working in this area over recent years. The ‘3R network’ in Baden-Wuerttemberg (<https://mwk.baden-wuerttemberg.de/de/forschung/forschungsfoerderung/3r-netzwerk-bw/>) is worth specific mention. Its members include:

- the 3R-Center for *In Vitro* Models, at the University of Tübingen (Prof. Dr Peter Loskill);
- the 3R-Center Rhein-Neckar, at CIMH Mannheim (Dr Marcus Meinhardt);
- the Interdisciplinary Centre for Gut Health Research, at the University of Heidelberg (Prof. Dr Beate Niesler);
- the 3R-US, at the University of Stuttgart (Prof. Dr Monilola Olayioye);
- Prof. Dr Ralf Watzlawick, at the University of Freiburg (Project on: Improving evidence and predictive value in experimental research);
- Prof. Dr Jan Tuckermann, at the University of Ulm (Project on: Refinement in complex stressful experiments on mice);
- Prof. Dr Karen Bieback, at the University of Heidelberg (Project on: 3Rs in the fields of tumour biology and drug testing);
- the 3R-BioMED Lab, at the University of Reutlingen (Prof. Dr Ralf Kemkemer);
- Leibniz Alternatives, at the Leibniz Research Institute for Environmental Medicine, Düsseldorf (Prof. Ellen Fritsche);
- TARCforce 3R, at the University of Mainz (Dr Nadine Baumgart);
- the Goethe University Frankfurt, with a Three Rs-focused professorship in the field of alternative testing (Prof. Maike Windbergs);
- the German Mouse Clinic, at the Helmholtz Centre Munich;
- the VZET (Virtual Centre for Alternative and Complementary Methods to Animal Testing), at the University of Veterinary Medicine in Hannover; and
- the SET Foundation, situated in Frankfurt/Main.

Below, we present further details on some of these, in particular giving an account of their founding histories.

BB3R: BB3R is the Berlin-Brandenburg research platform with integrated graduate education. It is a collaboration that was established in 2014 by researchers from FU Berlin, the University of Potsdam, Technical University Berlin, Charité–Universitätsmedizin Berlin, Zuse Institute Berlin and the Federal Institute for Risk Assessment (BfR). Many people from the different institutes were involved, but some played a particularly important role, namely: Prof. Monika Schäfer-Korting (FU Berlin); Prof. Johanna Plendl (FU Berlin); Prof. Günther Weindl (FU Berlin, now at the University of Bonn); Prof. Christa Thöne-Reineke (FU Berlin); Prof. Burkhard Kleuser (University of Potsdam); Prof. Axel Pries (Charité–Universitätsmedizin Berlin); Dr Robert Preissner (Charité–Universitätsmedizin Berlin);

Prof. Gilbert Schönfelder (BfR); Prof. Andreas Luch (Federal Institute for Risk Assessment); Dr Marcus Weber (Zuse Institut Berlin); and Prof. Roland Lauster (Technische Universität Berlin (TU Berlin)).

Since 2015, working groups at the Robert Koch Institute Berlin (under Dr Michael Beekes) and the Johannes Gutenberg–University of Mainz (under Prof. Jan Baumgart) have been associated with the BB3R, as well as further groups from FU Berlin (Prof. Gerhard Wolber, Dr Marie Weinhard, Prof. Lars Lewejohann and Prof. Wolfgang Bäumer), TU Berlin (Prof. Jens Kurreck) and Charité–Universitätsmedizin Berlin (Prof. Frank Buttgerit and Prof. Stefan Hippenstiel).

The BB3R combines excellent research at the Berlin-Brandenburg site and offers the world's only structured doctoral training according to international standards. BB3R's members are involved in various collaborations, and regularly present their research at national and international congresses. Contributions to the field — including scientific papers, master's theses and dissertations — have been recognised with numerous grants and awards. The seminar series *Alternatives to Animal Experiments in Research and Teaching*, which was organised by the BB3R, was awarded the Prize of the State of Berlin for the Promotion of Alternative and Complementary Methods for Animal Experiments in Education and Teaching in 2015.²⁵ Details can be found on the BB3R website: www.bb3r.de. This focus on the Three Rs resulted in the call for four related professorships: C. Thöne-Reineke, a new professorship of animal welfare, animal behaviour and laboratory animal science; S. Amasheh, with a focus on epithelial barriers; L. Lewejohann, adjunct professorship of refinement (with BfR); and Wolfgang Bäumer, as Full Professor of Pharmacology.

The FU Berlin has provided follow-up funding to the BB3R for their research, and has secured its future by appointing the successor of the BB3R spokesperson. After the retirement of Prof. Monika Schäfer-Korting in 2020, her successor Prof. Burkhard Kleuser took over the research in the field of *reduction* and *replacement*. As a former Professor in Potsdam, Prof. Kleuser represented the Brandenburg arm of the BB3R, and he has always been very involved in BB3R graduate training. His research focuses on studying the importance of biologically active lipids in stress responses and pathophysiological diseases. With his return to FU Berlin, it is ensured that research and training in the field of the Three Rs will continue there.

FU Berlin: FU Berlin research groups have successfully recruited third-party funds for Three Rs research (e.g. in the Excellence Cluster Science of Intelligence, BMBF funding). Furthermore, at FU Berlin, complex organ models of both normal and diseased states were developed for

different species, including humans. The morphological structural features of the models were mapped and evaluated for their predictivity potential in preclinical pharmacology, with a view to improving the current poor success rates in clinical studies. Moreover, within the framework of the BB3R (see above), precise information on the detailed and correct anatomy of the minipig large animal model — which is urgently needed by experimenters for the purposes of *refinement* — was developed with the help of computer tomography.

As part of the interdisciplinary, institution-overarching projects funded by Charité 3^R, FU Berlin researchers are investigating *refinement* strategies, for example in the project ‘Refinement — stress hormones and stress assessment’; in addition, the recently initiated ZIM project ‘RefineMon’ aims to enhance quality assurance and refinement of animal experiments. It is also noteworthy that FU Berlin has developed innovative products for implementing the Three Rs in teaching, by combining user analysis and cutting-edge techniques. For example, in the Bf3R-funded project ‘SimulRATor’, an interdisciplinary team of FU Berlin researchers is developing an anatomically correct and cost-effective simulator for use in laboratory animal science courses that is created at the Institute of Veterinary Anatomy by using 3-D printing techniques.

FU Berlin also collaborates with other Three Rs centres in Germany and the EU, such as the 3R Center at the University of Mainz and the newly founded 3R Competence Center at the University of Zurich through FU Berlin-UZH Joint Seed Funding. In addition, there is national and international co-operation with scientists in the field of the Three Rs and animal ethics. For example, the research project *Humanely ending the life of animals* is a collaborative partnership of scientists from the University of Zürich and FU Berlin, which is funded by the Federal Food Safety and Veterinary Office (FSVO, Switzerland).

CAAT-Europe: The Center for Alternatives to Animal Testing (CAAT) was founded in 1981.²⁶ CAAT-USA is part of the Johns Hopkins University Bloomberg School of Public Health, Baltimore, now with a European branch (CAAT-Europe), located at the University of Konstanz, Germany since 2010.²³ This transatlantic organisation, with ties to all parts of the world, promotes humane science by supporting the creation, development, validation and use of alternatives to animals in research, product safety testing and education. Internationally, CAAT for many years organised Three Rs centre meetings as satellites to world conferences, and maintained a joint platform on the AltWeb website (now transitioned to <https://caat.jhsph.edu/resources/index.html>).

CAAT seeks to effect change by working with scientists in industry, government and academia, to find new ways to replace animals with non-animal methods, reduce the

numbers of animals necessary or refine methods to make them less painful or stressful to the animals involved. This is promoted by regular workshops organised by its ‘transatlantic think tank for toxicology’ (t⁴). CAAT was initially funded by the US Cosmetic, Toiletries, and Fragrance Association (CTFA) with a \$1 million grant, but has since been supported by more than 50 companies and trade associations from various sectors, as well as philanthropic and public research funding. Over four decades, it expanded to all areas of animal use in industry, regulation and academia. Its work spans from proof-of-principle research into new alternatives funded competitively by various research funding bodies, to translational work of multi-stakeholder consensus processes, education and communication, as well as policy programmes, informing especially the US and EU legislative processes. Current focus areas with dedicated programmes include: microphysiological systems, pathway-based toxicology (the human toxome), good cell culture practice, evidence-based toxicology, green toxicology, refinement, *in silico* approaches (including read-across) and thresholds of toxicological concern, as well as integrated testing strategies.

CAAT-Europe was founded in 2010 to coordinate transatlantic activities around the development of new and improved methods in toxicology, to be a partner in strategy development, to provide platforms for different stakeholders, to exchange ideas and to support the Three Rs principles of humane science in different ways.²³ Dr Thomas Hartung serves as programme liaison representing Johns Hopkins, and Dr Marcel Leist serves as the University of Konstanz co-director. CAAT-Europe’s key areas are consumer protection, human health, new approach methodologies (NAMs), the Three Rs in general, toxicological testing strategies and systems toxicology, information and education on NAM and the Three Rs.^{27–39}

Charité 3^R: Charité 3^R (<https://www.charite3r.charite.de>) was founded in 2018 as an initiative of Charité–Universitätsmedizin Berlin, with the support of the Berlin Senate. It aims to support the implementation of the Three Rs at Charité–Universitätsmedizin Berlin and to establish Berlin-wide collaboration on the Three Rs. The idea of a Berlin Three Rs centre was put forward by several scientists from various biomedical research institutions in Berlin that were already associated with the BB3R, who initiated a *Memorandum for Rethinking Biomedical Research* in 2017. The founding of Charité 3^R was substantially driven by Axel R. Pries, dean of Charité–Universitätsmedizin Berlin, and was headed by Stefan Hippenstiel as spokesperson. Charité 3^R has so far organised two scientific Three Rs-related symposia — one in 2019 and one in 2021. An important milestone was the recent approval of funding for the Einstein Center 3R (<https://www.ec3r.org/>). This centre comprises eight biomedical research institutions from

Berlin, collaborating in Three Rs communication, education and 3-D tissue culture model development. The centre is currently funded for 3 years, with a €4.5 million grant by the Einstein Foundation Berlin. Charité 3^R closely collaborates with *Der Simulierte Mensch* (<https://www.si-m.org/>). This emerging research collaboration between Charité–Universitätsmedizin Berlin and Technische Universität Berlin harnesses both biomedical and engineering expertise to create human-based technologies for a new understanding of human health and diseases. In the context of the corona virus pandemic and future pandemic preparedness, Charité 3^R actively contributes to the German network Organo-Strat (<https://www.netzwerk-universitaetsmedizin.de/projekte/organo-strat>). This collaborative network consists of laboratories with expertise on organ models, virologists, infectiologists and other experts, with the aim of providing a rapid response for the stratification of organ involvement in COVID-19, as well as further infectious diseases of pandemic potential. This collaborative development of human-based models thereby provides an important contribution to the wider recognition of alternative methods.

ICAR3R: The Interdisciplinary Centre for 3Rs in Animal Research (ICAR3R) is located at Justus Liebig University (JLU) Giessen. Before ICAR3R was inaugurated, the Central Hessen Research Campus was founded in 2016 as an association of JLU Giessen, Philipps University Marburg and Technical University of Central Hessen (THM). The stated aim of the above alliance is to establish Central Hessen as a region of top-level research. At its heart is the joint development and expansion of selected research fields, including lung and infection research, psychology and neuroscience, as well as history and regional sciences. Part of the strategy is to strengthen animal welfare at these research sites, and beyond. To achieve their stated aim, the foundation of a centre for Three Rs research was initiated, providing two Three Rs-focused professorships — one in the field of *refinement* (in the Department of Veterinary Medicine at JLU Giessen) and one in the field of *replacement* (in the Department of Human Medicine at JLU Giessen).

In co-operation with the Philipps-University of Marburg, the THM and the Goethe University in Frankfurt, the ICAR3R aims to promote animal welfare in biomedical research and to develop concepts for the development of alternative research methods and husbandry conditions, in order to contribute to a sustainable reduction of laboratory animals. The interdisciplinary approach of ICAR3R is already reflected in its basic structure, with the centre's management being the responsibility of the two Three Rs-focused professors based in the departments of veterinary medicine and human medicine. Since the establishment of the centre, the Executive Director has been Univ. Prof. Dr med. vet. Stephanie Krämer, who holds the Professorship in

Laboratory Animal Science and Animal Protection, in the Faculty of Veterinary Medicine at JLU Giessen. The Deputy Director is Prof. Dr Peter Jedlicka, who holds the Professorship in Computer-based Modelling in the field of Three Rs Animal Protection, in the Faculty of Medicine at JLU Giessen. An important milestone was the kick-off symposium of the ICAR3R, *New Directions in Interdisciplinary 3R Research*, which took place on 18–19 October 2018 and welcomed almost 140 participants from seven countries. The event focused on the close linkage of the different scientific disciplines that aim to combine excellent research with animal welfare according to the Three Rs principles. The event showed that high-quality research and animal welfare can indeed go hand-in-hand. In October 2020, the centre welcomed almost 600 participants to the ICAR3R Symposium, which was followed by the ceremony for the Ursula M. Händel Animal Welfare Prize (€80,000), awarded by the German Research Foundation (DFG). Computer scientist, biochemist and medical scientist, Prof. Dr Thomas Hartung, from Johns Hopkins University in Baltimore and the University of Konstanz, Germany, as well as biochemist and toxicologist Prof. Dr Marcel Leist, also from the University of Konstanz, were the recipients of the 2020 Prize. By organising workshops on various topics (such as ethics or animal training), the ICR3R has also established itself as an important authority in Three Rs education.

Leibniz Alternatives: The Leibniz Alternatives research platform, at the IUF–Leibniz Research Institute for Environmental Medicine (IUF) in Düsseldorf, Germany, is a public–private partnership that was established in June 2020. It aims to initiate co-operation between industry and academic research to develop and use alternative methods to animal testing. Leibniz Alternatives draws on the IUF's 15 years of experience in the development and application of human-relevant alternative methods. The platform is headed by Prof. Dr med. Ellen Fritsche and operated by Dr Julia Tigges. Ellen Fritsche has been working at the IUF since 2001 and heads the research group on *Alternative method development for environmental toxicity testing* as a joint appointment with the Heinrich-Heine University in Düsseldorf. In 2018, she received the Ursula M. Händel Animal Welfare Award from the DFG. Her current research focus includes the development, scientific validation and application of 2-D and 3-D human cell-based assays for developmental and adult neurotoxicity, embryotoxicity and endocrine disruption, as well as their use in building adverse outcome pathways (AOPs).

By automating application and evaluation processes, Leibniz Alternatives makes sure that test methods are stable and reproducible for application within regulatory frameworks.⁴⁰ Under the umbrella of Leibniz Alternatives, several third party projects are currently in progress, among them: CERST-NRW (Center for Alternatives to Animal

Testing, funded by the Ministry for Culture and Science of the State of North-Rhine Westphalia, Germany); END-poiNTs and ONTOX projects (H2020); From (Screen) Hit to DNT Toxicant (DK-EPA); the screening of compounds in the developmental neurotoxicity *in vitro* battery (US National Toxicology Program); a project in the DFG Research Training Group 2578 dealing with the impact of genotoxins on the differentiation efficacy of murine and human stem and progenitor cells and functional competence of differentiated progeny derived therefrom; and a Cefic-LRI funded project aiming to fill the gaps of the current *in vitro* battery for developmental neurotoxicity (Cefic-LRI). Ellen Fritsche will also be involved in the Partnership for the Assessment of Risk from Chemicals (PARC, H2020) project. In view of this level of ongoing activity, it is clear that the reputation of the IUF in the Three Rs field was a key driving force behind the foundation of Leibniz Alternatives.

R2N: In order to expand the development of valid research models for science, and to meet the socio-political demand for the reduction and replacement of animal experiments, R2N was formed in 2017 as a research-driven Three Rs network in Lower Saxony (especially in the research-intensive region of Hanover-Göttingen-Braunschweig). Funded by the Lower Saxony Ministry of Science and Culture, its aim is to facilitate the development and systematic establishment of alternative methods in basic research. The collaborative partners have expertise in the fields of stem cell research, organoid technology, disease modelling, hypoxia, infection research and gene therapy. Prior to the establishment and funding of the R2N network, there was no coordinated association of participating institutions focusing on non-(living) animal biomedical research. Therefore, especially in the field of basic research, the formation of a clear strategy on innovative non-animal methods by a consortium focused on *replacement* and *reduction* was needed. In this respect, fundamental structural preparatory work had to be done and the collaborative research groups have clearly benefited from the coordination, interaction and networking. As a result of the R2N partnership, access to a range of extensive technologies and to human material were improved, test systems were further expanded or implemented, and the common focus of the network was significantly sharpened. Furthermore, it was possible to visibly position the network and its content-related activities at the local universities and beyond, which led to better awareness of the Three Rs principles at the various institutions. Young scientists, as well as the public, were informed about the importance of animal replacement methods in biomedical research, for example by showcasing the R2N at the Ideas Expo 2019.

The focus of the R2N so far has been on work modelling the digestive tract (oral mucosa, intestine and liver) and the upper and lower respiratory tract by using primary or adult

stem cells (AdSC), induced pluripotent stem cells (iPSC), complex culture conditions (air-liquid interface, hypoxia, chip systems) or precision-cut tissue sections. These systems have been established, further developed and optimised, and are already extensively implemented within the research projects of the various groups, as well as within other collaborative research associations. In this context, the expertise of the R2N research groups in genome editing and infection research was of particular importance. In addition, R2N groups have successfully developed animal-free systems for estimating the antigenicity of xenogeneic cell-free materials and for the safety assessment of vectors for gene therapy. Furthermore, expertise on preclinical systematic reviews was developed within R2N and applied to the R2N questions. Two ethics-focused working groups successfully considered the decision criteria that scientists use when choosing a model system, and the respective hurdles associated with their choice of using non-animal methods. Overall, the work resulted in numerous publications and the forging of strong links, both within and outside the network. This is reflected, for example, in the application of the systems in the context of COVID-19 research.⁴¹

TARCFORCE 3R: The Research Center for Animal Welfare and Laboratory Animal Science (TARCFORCE 3R) was founded in November 2019 in Rhineland-Palatinate as a spin-off of the Translational Animal Research Center (TARC) at the University Medical Centre in Mainz. Prior to the foundation of TARCFORCE 3R, the TARC had started to focus more and more on conducting Three Rs projects and implementing Three Rs methods. The TARC was already an associate member of the BB3R (Berlin, Germany), and TARCFORCE 3R was created in order to further expand this commitment to the Three Rs. Prof. Jan Baumgart (the head of TARC) and the Dean of the University Medical Centre were the key people involved in the creation of the centre.

TARCFORCE 3R has its own steadily growing research group focusing on *refinement*. As part of a large university animal facility, TARCFORCE 3R is aware of its responsibility toward laboratory animals, and thus it emphasises the importance of the welfare and protection of laboratory animals that are used in experiments, which currently cannot be replaced through the use of alternative methods. Nowadays, laboratory animal husbandry must offer more than optimal breeding and husbandry conditions. Therefore, a major interest at TARCFORCE 3R is to establish effective *refinement* strategies and to promote the use of the latest *refinement* methods.

3R-Center Rhine-Neckar: The Institute for Psychopharmacology, headed by Prof. Rainer Spanagel, at the Central Institute of Mental Health in Mannheim (CIMH), has a long-standing history of Three Rs activities. These

activities have been honoured by the European FISEA Award and the Ursula M. Händel Animal Welfare Prize, awarded by the German Research Foundation (DFG). To identify and systematically unlock further Three Rs potential, Prof. Spanagel and Dr Marcus Meinhardt designed a roadmap for future Three Rs activities at the CIMH. Due to its close proximity to basic and preclinical research sites, as well as the highly interdisciplinary nature of the science undertaken in the Rhine-Neckar region around Mannheim, the idea emerged to join forces and establish a common Three Rs centre between the CIMH, and the medical and biosciences faculties of Heidelberg University. The Rhine-Neckar region is located in the state of Baden-Wuerttemberg, which is home to several universities and non-university research institutions, and is one of the top biomedical science locations in Europe. This scientific power, however, also makes Baden-Wuerttemberg the German frontrunner in the use of laboratory animals. In turn, as an important biomedical research location, Baden-Wuerttemberg also bears a special responsibility for laboratory animal welfare. In recognition of this responsibility, the Ministry for Science, Research and Art Baden-Wuerttemberg, started a funding call in 2020 to establish a '3R Network Baden-Wuerttemberg'. In the scope of the ongoing Three Rs activities at the CIMH, it applied for funding to be part of the 3R Network Baden-Wuerttemberg, and to establish a Three Rs centre in the Rhine-Neckar region. In January 2021, the necessary funding was granted to be able to start work on the creation of the '3R-Center Rhine-Neckar'.

VZET: The Virtual Center for Alternative and Complementary Methods to Animal Testing (VZET—*Virtuelles Zentrum für Ersatz- und Ergänzungsmethoden zum Tierversuch*) was founded in 2009 at the University of Veterinary Medicine Hannover (TiHo). The founding board included Prof. Dr Pablo Steinberg as chairman, Prof. Dr Manfred Kietzmann as deputy chairman, as well as Prof. Dr Gerhard Breves, Prof. Dr Christiane Pfarrer, Prof. Dr Silke Rautenschlein and Prof. Dr Karl-Heinz Waldmann. TiHo scientists develop, optimise and/or validate alternative and complementary methods to animal testing. The VZET facilitates the combination of Three Rs activities at the various institutes in research and teaching, by means of interdisciplinary co-operation. The key focus, besides widely used human or murine models in the One Health context, are also various *in vitro* animal models that mimic pathological findings, infection and inflammation in companion animals. Since its inception, three scientific symposia (in 2010, 2012 and 2019), with international keynote speakers from the Three Rs field, have been successfully organised on important One Health topics, and these should continue in 2022.

Ireland

The concept of the Three Rs started to gain momentum in the Republic of Ireland after *Directive 2010/63/EU* was transposed into the SI 543 of 2012, and adopted in January 2013. There is still a long way for the Three Rs to be fully embraced by the Irish scientific community, as there are still misunderstandings on what they are in relation to animal research. In order to address this and engage the Irish scientific, educational and regulatory communities into the active uptake of the concept, a group of experts from Trinity College Dublin are now actively working towards the aim of establishing the first Irish Three Rs centre.

Trinity College Dublin (TCD): Currently, a Three Rs centre does not exist in the Republic of Ireland. Nevertheless, an expert group comprised of Viola Galligioni, Daniel Ruiz-Pérez, Dania Movia, Adriele Prina-Mello and José M. Sánchez-Morgado have been actively working over the last decade, toward the aim of filling this gap. In August 2016, José M. Sánchez-Morgado started an initiative to establish the first Three Rs centre in the Republic of Ireland, after discussions with Syrcle (<https://www.syrcle.network>) about the reproducibility issues in animal research, in preparation for the 2017 LAVA-ESLAV-ECLAM Conference on the Reproducibility of Animal Studies. After the meeting, at the round-up session on improving uptake of the ARRIVE Guidelines,⁴² further discussions followed as to how to establish a Syrcle Centre in Ireland. During 2018, these conversations resulted in what would be the first Trinity College Winter School on Systematic Reviews, held in January 2019. Furthermore, in 2019, Viola Galligioni joined the Education and Training Platform for Laboratory Animal Science (ETPLAS, <https://etplas.eu/>) Working Group 1 (Development of guidance on the principles and quality standards of assessment criteria for learning outcomes) and Working Group 2 (Development of assessment criteria for Core modules and Function A specific modules (ethics)). During 2019 and 2020, the working groups produced a Guidance Document to enable a harmonised assessment of competence for carrying out procedures on animals (Function A persons),⁴³ according to Article 23 of *Directive 2010/63/EU*.

Also, in 2019 TCD joined the Culture of Care (CoC) Network. The CoC Network represents an opportunity to disseminate results, ideas and knowledge in a fast and efficient manner, and also to provide a forum for exchanging ideas and receiving and providing help. In 2020, the CoC network decided to create subgroups, to manage more efficiently the discussion of various topics. TCD is involved in the subgroup 'Anaesthesia, care, welfare, severity, humane endpoint'. The subgroup is currently working on best practices that could be shared with the scientific community. During 2020, Daniel Ruiz-Pérez started an educational

programme on the Three Rs for all the scientists working with animals at TCD. This initiative is now going to a national Irish level through Trinity's virtual learning environment. During these years, Dania Movia and Adriele Prina-Mello at the TCD Laboratory for Biological Characterisation of Advanced Materials (LBCAM) have extensively liaised with the EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), an integral part of the European Commission Joint Research Centre (JRC), whose mandate includes a number of duties to advance the *replacement, reduction and refinement* of animal procedures in research. In 2019, Dania Movia was appointed member of the Scientific Advisory Panel of Animal-Free Research UK, a renowned medical charity set up 50 years ago and still at the forefront of awarding grants to scientists, with the aim of supporting the replacement of animal use in medical research.

During November 2019, José M. Sánchez-Morgado approached Norecopa, to further discuss how TCD could establish a Three Rs centre in Ireland. In December 2019, the Comparative Medicine Unit at Trinity College Dublin joined the European Consensus Platform for Alternatives (ecopa), as an associate member. In 2020, the communications with Syrcle continued, but due to financial constraints the initiative to establish the Irish arm of Syrcle (iSyrcle) did not progress. In February 2021, Viola Galligioni, Daniel Ruiz-Pérez, Dania Movia, Adriele Prina-Mello and José M. Sánchez-Morgado started an initiative, with the aim of establishing the Irish Consensus Platform for Alternatives (Icopa), by holding talks with the Irish competent authority, the Health Products Regulatory Authority (HPRA), the Irish Society for the Prevention of Cruelty to Animals (ISPCA) and Charles River. Activities for the creation of the iSyrcle are also still ongoing, and the initiative may take full shape in early 2022. It is hoped that these two initiatives together will result in the formation of the first national Three Rs centre in Ireland, based at TCD.

Italy

Animal experimentation is hotly debated by the Italian public, and animal rights groups are vociferous and active, as are the equivalent pro-testing groups. *Directive 2010/63/EU* was eventually transposed into national law in 2014, with Italy being the last country to transpose and implement the Directive after heated debates in Parliament, as well as in general society, between supporters and opponents of animal testing. It is worth noting that, in some aspects, the Italian Law goes even further than the Directive, dealing with, for instance, xenotransplantation and substances of abuse, even though the Directive makes no mention of these. Moreover, it imposes a ban on the breeding of cats, dogs and non-human primates for basic research. The clauses regarding the ban on the use of animals for

experiments on xenotransplantation and substances of abuse have not yet been implemented. The role of Centro 3R is to provide a balanced view, acknowledging the importance of animal research and its refinement and reduction, as well as the importance of scientific and technological progress to improve the efficacy of current methods, bearing in mind the Directive's ultimate goal. In addition, IPAM (Italian Platform for Alternative Methods; <https://www.ipamitalia.org/>) is a private–public platform and its work is dedicated to the Three Rs cause.

Centro 3R: Centro 3R stands for 'Centro Interuniversitario per la promozione dei principi delle 3R nella didattica e nella ricerca 3R' (Interuniversity Centre for the Promotion of 3Rs Principles in Teaching and Research). It was founded in December 2016 by the University of Pisa and University of Genova, to promote the Three Rs at university level. It was started by Prof. Arti Ahluwalia and Prof. Anna Maria Bassi, who had both been involved in an expert team set up by the Italian Ministry of Health to implement legislation on the protection of animals for scientific purposes. In the first year, the Polytechnic of Milan and the Polytechnic of Torino joined Centro 3R. Other universities joined in quick succession, namely: the University of Pavia, the University of Milan-Bicocca, the Campus-Biomedico of Rome, and (most recently) the University Polytechnic of the Marche (ex-University of Ancona). Centro 3R was set-up to support the practical implementation of the legislation and to encourage the adoption of the Three Rs concepts in teaching and basic research at all levels. It embraces all of the Three Rs, and actively promotes the concept as being synonymous with responsible, rigorous and high-quality research. Its specific aims are to:

- promote the establishment of courses focused on the Three Rs within the core curricula of scientific degree programmes;
- create a multidisciplinary open e-infrastructure, to include resources to facilitate the teaching of the Three Rs principles, news and links for researchers to find Three Rs-related resources, an internal platform to promote the sharing of animal tissues, and a system for rehabilitating laboratory animals;
- promote scientific and cultural debates;
- highlight, develop and coordinate interdisciplinary studies in the context of the Three Rs;
- implement, promote and develop new integrated *in silico* and *in vitro* methods;
- organise courses, seminars, refresher courses and meetings within various scientific disciplines;
- establish prizes and fellowships for young researchers, to encourage Three Rs studies;
- encourage collaboration with Animal Welfare Bodies to favour the adoption of alternative methods;

- promote technology transfer and translational research; and
- encourage national and international collaborations.

Over time, members of Centro 3R have developed new teaching programmes and specific teaching modules on the Three Rs, which are now offered to students at undergraduate and postgraduate level. Currently, Centro 3R has over 400 members, including eight universities (the Universities of Pisa, Genova, Pavia, Milano-Bicocca, Polytechnics of Torino and of Milan, the Campus-Biomedico in Rome, University-Polytechnic of Le Marche and the National Institute for Meteorological Research) and one research institute.

Luxembourg

Luxembourg has established procedures for the implementation of the Three Rs principles, as project applicants who intend to carry out animal experiments have to carefully describe these aspects. Luxembourg has a representative in EURL ECVAM's Network for Preliminary Assessment of Regulatory Relevance (PARERE) at the Joint Research Centre (JRC), and institutions from Luxembourg have submitted their first applications for test validation to EURL ECVAM. Due to the small size of the country, there will only be one Three Rs centre in Luxembourg.

Luxembourg 3Rs Platform: Preparatory meetings with national stakeholders (ministries, academia and industry) began in 2021. Thus, the platform is currently in the development and mapping phase. However, a national 'Three Rs Day' did take place in Luxembourg, in 2019.

Netherlands

The National Centre for Alternatives to Animal Testing (NCA) was launched on 1 February 1994. The centre was financially supported by the 'Platform Alternatieven voor Dierproeven' (Platform Alternatives to Animal Testing; a partnership of government, animal protection organisations and industrial companies that conduct animal experiments). The NCA was housed at the Faculty of Veterinary Medicine and worked closely with the Department of Laboratory Animal Science at Utrecht University. At that time, Utrecht University was already at the forefront of humane animal testing and alternatives. The NCA was officially inaugurated in Utrecht on 10 June 1994, during a short symposium. In 1999, the Platform ended its support of the NCA. After this, in 2000, the collaboration with ZON (Zorgonderzoek Nederland) began, which lasted until 2008 (two project rounds). At the same time, a chair in alternatives to animal testing was established at Utrecht University, and based at

the NCA. In 2009, the situation changed drastically. The support from ZON had ended, and the then-Ministry of Health, Welfare and Sport decided to transfer the Three Rs efforts to the National Institute for Public Health and the Environment (RIVM). Legally, the new NKCA (National Knowledge Centre for Alternatives to Animal Experiments) was a partnership between the RIVM and Utrecht University, and included the diminished workforce of the former NCA.

In addition to the establishment of national centres, such as the Netherlands Centre for Alternatives to Animal Use (NCA), initiatives have also been taken in various European countries at the local level, to develop and implement Three Rs alternatives in a coordinated and structured manner. These 'local centres' are often affiliated with existing research institutions where animal experiments are carried out. In the Netherlands, the RIVM was the first institution to establish a local Three Rs centre, in 1992. The Coordination Point for Alternatives to Animal Testing (CAD), as the centre was called, worked under its own capacity and had a modest budget. Research at the RIVM focused mainly on public health topics, such as vaccinology, pharmacology and toxicology. The CAD had two coordinators and an advisory committee consisting of respected researchers from the institute. Accountability for activities was provided annually to the management of the RIV(M). In the Netherlands, the concept of local centres has subsequently been adopted by various institutions, both academic and industrial. Looking at the local initiatives, it is clear that institute-based centres offer additional opportunities and possibilities that are less readily achieved by national centres, for example:

- a commitment from the management and policy officers that subsequently contributes to the implementation of a culture of care within the institute;
- short lines of communication with researchers and biotechnicians that facilitate a rapid response to current events, the direct involvement of institute staff and a quick start to Three Rs projects;
- a fertile ground for the effective implementation of Three Rs alternatives in ongoing research projects;
- organisation of local meetings with experts from outside the institute, which optimises the transfer of information, ensures topicality and makes it possible to identify cross-references within the research.

Within the RIVM, the CAD has contributed to improving animal welfare (for example, by identifying humane endpoints in research that involves significant distress), implementing *reduction* and *refinement* by replacing challenge tests in vaccine control with less burdensome and less animal-intensive serological methods, and implementing *replacement* strategies (for example, replacing the ascites

method for monoclonal antibody production with *in vitro* methods).

Since 2018, the Dutch government has been working with several partners on the Transition Programme for Innovation without the use of animals (TPI). TPI's partners are active in government, society, academia and the business community. As long as animal tests are needed in several domains, the Three Rs are important for the welfare of the animals involved. The question that the TPI wants to answer is not: *Is this animal-free method good enough to replace my animal experiment?* but rather *Which method provides the best answer to the research question?* New innovative strategies are necessary to find methods that better translate to humans, and that help patients with personalised medicine. The approach of the partners in the TPI programme is to build up animal-free innovations rather than emphasise the reduction of animal tests. The TPI partners are also working together to achieve the ambition of making the Netherlands a frontrunner in the international transition of innovation without the use of animals. The mission is to develop animal-free models and tests that better predict the effectiveness and safety of medicines and substances, thus making animal procedures increasingly redundant. One of these TPI's partners is NCad.

NCad: NCad is the Netherlands National Committee for the protection of animals used for scientific purposes. On 18 December 2014, EU *Directive 2010/63/EU* on animal experiments in scientific research was implemented in the national legislation of the Netherlands. This resulted in new tasks, competences and relations. One requirement of the European Directive is that each Member State must appoint a national committee for the protection of animals used for scientific purposes. In the Netherlands, NCad has been engaged in improving the welfare of laboratory animals since 18 December 2014. NCad's goal is to achieve noticeable improvements that are specifically related to the Three Rs of animal procedures, and to the associated ethical review in scientific research (including applied scientific research) and teaching. NCad aims to minimise laboratory animal use, both at national and international level, and it is a key player in the area of animal procedure policy, bringing parties together and sharing knowledge with a view to furthering the development of laboratory animal policy and the implementation of Three Rs policy (including ethical review). This is based on the NCad's meticulously substantiated advisory reports (and policy advisory reports). NCad paves the way for a responsible animal procedure policy for all concerned in the Netherlands, and launches initiatives to promote innovation, as a result of which it achieves tangible results, both at national and international level. NCad will achieve its goal, in accordance with its statutory mandate, by submitting solicited and

unsolicited advisory reports to the Minister of Agriculture, Nature and Food Quality, the Central Authority for Scientific Procedures on Animals (CCD) and the Animal Welfare Bodies (IvD) regarding:

- the acquisition, breeding, housing, care and use of animals in procedures, as well as about alternatives to animal procedures;
- the dissemination of best practices for the use of animals in experimental procedures;
- the exchange of information with national committees of other Member States about the IvDs' performance, the assessment of project proposals, and the dissemination of best practices within the EU;
- the promotion of the development, validation, acceptance and use of alternatives to animal procedures, both at national and international level;
- the best ways in which to support communication with (and between) professionals in the field of animal procedures and alternatives, as well as how to best provide details about animal procedures and alternatives to the general public.

3Rs-Centre of the Utrecht University and University Medical Centre Utrecht: This organisation started in 2010 as 3Rs-Centre Utrecht Life Sciences, when the Netherlands Centre Alternatives to Animal Use (NCA, 1994–2010) was dissolved. It was a partner in the Netherlands Knowledge Centre on Alternatives to Animal Use (2010–2014). The activities at Utrecht University (UU) focused on the field of Three Rs education, training and communication. In order to allow the Three Rs activities within UU to keep their own identity, the relevant Utrecht department was renamed the '3Rs-Centre'. The collaboration with RIVM ultimately lasted four years. From 2015 to 2018, a number of activities, in particular in the field of communication, education and training, were continued for the Ministry of Agriculture, Nature and Food Quality. In the meantime, since 2015, the expertise of the 3Rs-Centre has been partially seconded to the Animal Welfare Body-Utrecht (AWB-Utrecht).

Norway

The implementation of the Three Rs in Norway has a long history. Veterinarian Stian Erichsen, who served both as Secretary General and President of the International Council for Laboratory Animal Science (ICLAS), was one of those who developed Laboratory Animal Science as a discipline in Norway from the early 1960s onwards,⁴⁴ following several study visits to the UK during the period when Russell and Burch were launching their Three Rs tenet. He was responsible for building the first animal facility that followed these modern principles, and he was also actively involved in the construction of the European Convention

ETS 123, leading the committee during the final stages before it was completed in 1986. This work stimulated the recruitment of several other leading veterinarians who helped develop the laboratory animal community in Norway, and who have held many courses in Laboratory Animal Science, with emphasis on the Three Rs, especially from 1985 onwards. In Norway, Norecopa is the only official Three Rs centre, but many research institutions have groups of scientists working actively on Three Rs-related projects.

Norecopa: This platform was founded in 2007. Following the creation of ecopa, the Norwegian Ministry of Agriculture and Food set up a working group in 2000, to investigate the need for a National Platform for Alternatives in Norway. The group concluded in 2001 that such a Platform should be established. Then, a Governmental Action Plan (No. 12 (2002–2003)) on Animal Welfare and Animal Husbandry stated that the animal research community in Norway needed a National Platform that could work to advance alternatives to animal experiments.⁴⁵ The Report also recommended that a State Fund for research and development of alternatives was set up, but this was never established. A temporary platform was run by the Food Safety Authority in 2005–2006, and then, on 10 October 2007, Norecopa was established, funded jointly by the Ministry of Food and Agriculture, and the Ministry of Trade, Industry and Fisheries. Norecopa's secretariat is, for technical budgetary reasons, affiliated to the Norwegian Veterinary Institute. Norecopa is, however, an independent member organisation, with a Board representing all the four major stakeholders (regulators, industry, research and animal welfare), and the Annual General Meeting representing the highest authority. Since the Board includes representatives of all the stakeholders, Norecopa satisfies the conditions for membership of ecopa, and has been a member since its foundation. Norecopa has its own statutes.⁴⁶

Poland

In Poland, several individuals and institutions are currently actively involved in the Three Rs, notably the:

- Ministry of Education and Science (Anna Passini, Chief Specialist, Warsaw);
- Bureau for Chemical Substances (Agnieszka Duda, President, Lodz);
- Łukasiewicz Research Network–Institute of Industrial Organic Chemistry, Pszczyna Branch;
- National Centre for Alternative Methods to Toxicity Assessment (CMA), at the Nofer Institute of Occupational Medicine (NIOM), in Lodz; and

- Selvita S.A. Cell Biology Laboratory, at Park Life Science in Krakow.

The CMA and Selvita are Polish laboratories that are members of the European Union Network of Laboratories for the Validation of Alternative Methods (EU NETVAL). The history of the Three Rs in Poland is closely linked to the CMA.

CMA: In the years 1992–1994, NIOM carried out the project 'Verification and standardisation of selected *in vitro* methods used to assess the toxic effect of chemical substances' (as part of the Chemical Safety project financed by the Polish Scientific Research Committee, coordinated by Prof. Konrad Rydzyński). To identify the scope of application of *in vitro* alternative methods in Poland, a questionnaire was prepared and sent to medical academies, institutes and other research units. In 1997, NIOM was actively involved in the implementation of the long-term Strategic Government Programme 'Human Health and Safety in the Work Environment', supported by the Ministry of Health. As part of this programme, several projects concerning alternative methods were undertaken by NIOM and the Central Institute for Labour Protection. In 1999, the National Centre for Alternative Methods to Toxicity Assessment (CMA) was established at NIOM within the complementary programme financed by the Ministry of Health. The initial goals and tasks of CMA were:

- promoting the idea of using alternative methods for toxicity assessment (the Three Rs concept);
- collecting and distributing data on the concept and application of alternative methods;
- promoting co-operation between research centres employing alternative methods;
- exchanging information on relevant national and international research projects; and
- providing information on grants and the potential application of alternative methods, both in Poland and abroad.

During 1999–2002, the free-of-charge quarterly, official journal of the CMA, *Vitryna*, was published. The journal described and disseminated information on alternative methods. In 2003, Dr Maciej Stepnik, the head of the CMA at that time, approached Prof. Vera Rogiers, president of ecopa. The CMA was undertaking numerous attempts to create a Polish Platform (Polcopa) with partners from the four stakeholder fields (government, industry, science and animal welfare), which is a prerequisite for ecopa membership. Successful contacts had already been established with several stakeholders (from government: the Bureau for Chemical Substances, National Toxicological Information Centre (Central Register of Cosmetics), Department of Food

Hygiene, Nutrition and Objects of Use, and the Chief Sanitary Inspectorate; from industry: Dr Irena Eris Cosmetic Laboratories and the Institute of Organic Industry in Pszczyna (presently known as the Łukasiewicz Research Network–Institute of Industrial Organic Chemistry, Pszczyna Branch); from science: National Commission at the International Council for Laboratory Animals Science (ICLAS), Clinic of Transplantology and Central Tissue Bank, Medical University of Warsaw, Centre for Medical Biology and Microbiology, and the Polish Academy of Sciences; from animal welfare: Polish Society for the Protection of Animals and ARKA Animal Protection Society). The main aims of Polcopa were also defined, namely:

- to identify national requirements surrounding the use of alternative methods in various fields of research (e.g. by distributing a questionnaire to major laboratories, creating the Polcopa website and the ‘e-mail news network’, publishing guides and brochures, etc.);
- to develop and implement a national education/information programme promoting alternative methods (via seminars, courses, lectures, multimedia presentations, etc.);
- to assist concerned laboratories in implementing alternative methods;
- to undertake close scientific co-operation with other platforms, such as ecopa and ECVAM;
- to facilitate the exchange of problem-solving experience through co-operation between groups;
- to communicate with the scientific community and the media, etc.; and
- to co-operate with, and provide substantive support for, the authorities responsible for the legal aspects relating to animal experiments.

The initial meeting of Polcopa’s partners took place on 21 September 2004, at the Dr Irena Eris Science and Research Centre, with a dozen representatives mainly from the cosmetics industry and science. Following this, on 16 November 2004, a meeting of over 30 Polish partners of Polcopa with Prof. V. Rogiers was organised by Dr Dębowska (representative of Dr Irena Eris Science and Research Centre), Dr Stepnik (Head of CMA) and Dr Śladowski (member of the EURL ECVAM Scientific Advisory Committee (ESAC)), which also took place at the Dr Irena Eris Science and Research Centre. Following on from this meeting, the first Polcopa activities were undertaken in 2007, although many of the platform’s aims were not eventually achieved. The initial activities mainly comprised a series of lectures and participation at several conferences, held between March and November 2007. Work on the preparation of Polcopa’s statutes was subsequently initiated, but unfortunately, due to the lack of sufficient number of

members at that time, Polcopa’s activities were not officially enshrined in law. Consequently, after four years of work, the activities of Polcopa were suspended.

In 2008, NIOM obtained a GLP certificate as the first laboratory in Poland employing alternative methods. All *in vitro* studies at NIOM are currently performed at the Department of Translational Research (formerly the Molecular Toxicology Laboratory, ZTM), within the remit of the CMA. In December 2015, the Molecular Toxicology Laboratory NIOM/CMA was invited to join EU NETVAL. As a member of EU NETVAL, it participated in a pre-validation study to implement/establish a new method for the detection of potential thyroid disruptors (the T-screen using the GH3 cell line). In addition, the CMA is also working to implement an *in vitro* method for the assessment of skin irritation by using a reconstructed human epidermis model (as another study performed in accordance with GLP standards within the remit of the CMA). In the previous years, nanotoxicology studies were undertaken at the Molecular Toxicology Laboratory NIOM/CMA as new challenges in the Three Rs field. The focus was the evaluation of chemicals in the nano-form, for which established *in vitro* and *in vivo* toxicological methods might have to be modified and improved to meet Three Rs criteria. This expertise is reflected in the centre’s participation in several projects, such as: NANO-INTERACT (the development of a platform and toolkit for understanding interactions between nanoparticles and the living world (2007–2009)), NANOGENETOX (the safety evaluation of manufactured nanomaterials by characterisation of their potential genotoxic hazard (2010–2013)), and MARINA (managing the risks of nanomaterials (2011–2015)).

In 2018–2019, a platform for co-operation on alternatives with the National Ethics Committee on Animal Experimentation and other Polish stakeholders (the National Coordinator on Research Methods, Inspector of Good Laboratory Practice, Bureau for Chemical Substances, Nofer Institute of Occupational Medicine, National Centre for Alternative Methods for Toxicity Assessment, and the Chief Sanitary Inspectorate) was established. The platform will soon be resumed, after some remaining organisational changes are actioned.

Portugal

Three Rs activity was almost inexistent in Portugal until the foundation of SPCAL–Sociedade Portuguesa de Ciência em Animais de Laboratório (<https://www.spcal.pt/en>) in 2003. During the first decade of the 2000s, the infrastructure supporting animal research in Portugal was subject to intense development, with many animal facilities being set-up or extensively restructured and developed. This was accompanied by an increase in qualified personnel working in

refinement. Further key milestones in this history were: the first SPCAL congress in 2009, the establishment of the Network of Portuguese Animal Welfare Bodies ‘Re-deORBEA’ in 2015, and the election of Ana Isabel Santos as the first FELASA president from Portugal. Around 2008–2010 there was a peak in political and societal discussion concerning animal experimentation, which was sparked by the announcement of a plan to build a large-scale animal research facility to serve all the research institutions in the Lisbon region. In this context, in 2010, the Portuguese Parliament adopted a resolution (*Resolução da Assembleia da República n.º 96/2010*, <https://dre.pt/application/conteudo/343104>) recommending several measures to promote the Three Rs. Since then, several of these measures have been at least partly put in place, as a result of the transposition of *Directive 2010/63/EU* and its implementation within *Decreto Lei 113/2013*. However, the recommendation to create a structure with the competences of a Three Rs centre has never materialised. Apart from research institutions, the Sociedade Portuguesa para a Educação Humanitária (SPEDH; founded in 2006 as the Portuguese partner of the Jane Goodall Institute programme, Roots & Shoots) organised two sessions of the International Conference on Alternatives to Animal Experimentation, in 2014 and 2015, but visible activity in the Three Rs field has not been maintained since.

Within the research community, three institutions present concerted efforts in the Three Rs field:

- Firstly, the Consortium for Genetically Tractable Organisms (CONGENTO) (<https://congento.org/>), which has a notable Three Rs component to their work. Based in Lisbon, CONGENTO is a collaboration between four leading research institutions in biomedicine and fundamental biology, providing support infrastructure for research with non-human animal models. The Three Rs are primarily represented by *refinement* initiatives, through the provision of training and expertise to support researchers working with animal models.
- Secondly, in terms of research on *replacement*, the work of the biomolecular diagnostics group at the Instituto de Tecnologia Química e Biológica António Xavier (ITQB) at the Universidade NOVA de Lisboa (<https://www.itqb.unl.pt/labs/biomolecular-diagnostic/group-members>) is highly significant. Their work has received international recognition through the PETA (People for the Ethical Treatment of Animals) International Science Consortium Ltd early-career scientist award in 2019, which was presented to PhD student Patricia Zoio.
- Thirdly, the i3S, which represents the first fully-fledged Three Rs centre in Portugal. Its recent

founding history and further details of the important work that it has planned for the future are outlined in the next section.

i3S: Founding of the Portuguese Three Rs centre, i3S, was instigated in 2021. The basis of its foundation is the commitment to Three Rs engagement and the strong research tradition in bioengineering, present at i3S and at its founding institutions (the Institute of Molecular and Cell Biology (IBMC) and the Institute of Biomedical Engineering (INEB), in Porto). The initiative has been led by Dr Anna Olsson, with key support from the directors (Alexandre Quintanilha, Claudio Sunkel and Mário Barbosa), and from the named veterinarians (Isabel Carvalho, 2004–2010; and Sofia Lamas 2010–present).

With over 1000 researchers and nearly 300 PhD students, i3S is the biggest biomedical and basic biology research institute in Portugal. The centre was formed in 2008 as a consortium of three existing institutes: IBMC, INEB and the Institute of Pathology and Molecular Immunology at the University of Porto (IPATIMUP). The Three Rs centre is part of the i3S strategic plan for 2021–2025. A few key milestones in the development of the Three Rs history of the institute are: in 2002, the foundation of the first animal ethics committee by Prof. Alexandre Quintanilha; in 2005, the establishment of a training programme (FELASA-accredited in 2008) and a research group in Laboratory Animal Science by Dr Anna Olsson; in 2018, AAALAC-International accreditation of the animal facility, in an effort led by Dr Sofia Lamas; and in 2019 an H2020 grant to set-up competence in organoid and other advanced 3-D models, to Dr Anna Olsson, Dr Margarida Saraiva, Dr Cristina Barrias and Dr Celso Reis.

Romania

ROCAM is currently the only Three Rs centre in Romania.

ROCAM: The Romanian Center for Alternative Test Methods (ROCAM) was established in June 2015, with the main goal of supporting and promoting the Three Rs principles (*Replacement*, *Reduction* and *Refinement*) in Romania. ROCAM was coordinated at the beginning by Prof. Carmen Socaciu, with Dr Lucian Farcal playing a very important part in the initiation and promotion of the centre. From the very start, the centre was divided into three working groups (WGs): the *replacement* WG, coordinated by Prof. Adela Pintea; the *reduction and refinement* WG, coordinated by Prof. Bogdan Sevastre; and the *education and training* WG, which is the most active and is supported by Dr Dumitrita Rugina, Dr Orsolya Sarpataky, Dr Zorita Diaconeasa, Dr Andras Nagy and Dr Flaviu Tabaran.

ROCAM is hosted and supported by the University of Agricultural Sciences and Veterinary Medicine in Cluj-

Napoca at the Institute of Life Sciences. In March 2017, ROCAM became Associate Member of ecopa. ROCAM promotes the application of alternative methods in industry and their acceptance by Romanian regulators, and also the development of new methods and approaches. ROCAM acts as a bottom-up and top-down hub, in order to disseminate information on Three Rs approaches. ROCAM provides and facilitates training and educational programmes in the area of the Three Rs, as well as supporting research activities for the development, optimisation, validation and application of alternative methods to animal testing (e.g. *in vitro* and *in silico* models). Since 2015, ROCAM has hosted many conferences and workshops, such as: in November 2015, a toxicology and Three Rs workshop led by Prof. Francesca Caloni (Università degli Studi di Milano); in May 2016, a lecture on ‘Alternative methods to animals testing used in toxicological studies’; also in May 2016, CAAT Academy training on ‘*In silico* modelling and tools under REACH’; in February 2017, a ROCAM-IPAM-CELLTOX workshop ‘*IN vitro* toxicology: from INtestine to braIN’; in September 2017, a workshop on artificial skin models as replacement methods, held during the Conference of the National Society of Pathophysiology; and also in September 2017, a session on ‘Advanced *in vitro* cell culture workshop and training’.

Members of ROCAM are frequently involved in international and national events, such as the First Meeting of European 3Rs Centres, held in April 2015 (Ispra, Italy); the 19th European Congress on Alternatives to Animal Testing (EUSAAT), held in September 2015 (Linz, Austria); the first National Toxicology Congress, held in October 2015 (Bucharest, Romania); the 3Rs Centres meeting, held in May–June 2016 (Ispra, Italy); the JRC Summer School on ‘Alternative approaches for risk assessment’, held in May 2017 (Ispra, Italy); the ecopa SSCT workshop, held in June 2017 (Helsinki, Finland); the EuroNanoForum, in June 2017 (Valletta, Malta); the second National Congress of Toxicology, held in June 2019 (Bucharest, Romania); the 14th FELASA congress, in 2019 (Prague, Czech Republic); and the ecopa General Assembly, in November 2020 (online). The last two years were significantly influenced by the COVID-19 pandemic, thus slowing down ROCAM’s activity. However, ROCAM is determined to resume normal activities as soon as the pandemic-related situation returns to normal. The plan is to continue education and training in the area of *replacement*, *reduction* and *refinement*, and expand the education framework in the area of Laboratory Animal Science, by promoting the responsible and ethical use of laboratory animals according to current legislation.

Slovak Republic

A number of universities and several institutes of the Slovak Academy of Sciences had already started to use *in vitro* cell

culture techniques during the Czechoslovak era. One of the pioneers, who brought these techniques to Slovakia and significantly contributed to their practical implementation, was Ivan Stanek. His early work was an inspiration to many of his pupils and followers. *In vitro* cell cultures were also used by the Slovak pharmaceutical industry. For example, by the late 1950s, the IMUNA company, founded in 1953 in Šarišské Michalany, recognised the benefits of *in vitro* techniques in the production of vaccines and biologicals. The use of cell cultures in research was mainly due to their scientific and practical benefits, rather than ethical considerations. The Three Rs concept was recognised in Slovakia much later, mainly in connection with the legislative changes made during the process of joining the EU. These changes included the ban on testing cosmetic products in animals, the acceptance of validated alternative methods into EU legislation and regulatory toxicology, and the requirements of *Directive 86/609/EEC* on the protection of animals used for experimental and other scientific purposes. When Slovakia joined the EU, in 2004, the number of animals used for experimental purposes started to decline.

The international symposium on the promotion of the Three Rs concept in Slovakia, Slovenia and the Czech Republic, which took place in Prague in 2001, greatly increased understanding of the importance of alternative tests and the need for their independent validation. Discussions also focused on the implementation of the Three Rs in basic research and education. Slovakia was represented on the scientific programme with four lectures from the Slovak University of Technology, the Slovak Academy of Sciences and the private pharmaceutical sector. The concept of the Three Rs was established in Slovakia mainly due to the implementation of the EU law on Cosmetics, banning the testing of cosmetic ingredients in animals and further enforced by *Directive 2010/63/EU* on the protection of animals used for scientific purposes. In Slovakia, the most active organisation in relation to toxicology is the Slovak Toxicology Society (SETOX), which was established in 2006. SETOX is a member of the Federation of European Toxicologists and the European Societies of Toxicology (EUROTOX) and is also responsible for the ERT register of Slovak toxicologists. SETOX has four divisions, namely: experimental toxicology, clinical toxicology, industrial toxicology and *in vitro* toxicology. The *In Vitro* Division (IVD-SETOX) was established by Helena Kandarova in 2011 and became a platform to promote the use of *in vitro* methods within the SETOX network. The mission of IVD-SETOX is:

- to promote *in vitro* toxicology and its development;
- to support the practical implementation of *in vitro* methods;
- to stimulate, support and promote the use of *in vitro* tests in the field of education;

- to serve as an information channel between national and international groups interested in toxicology *in vitro*; and
- to organise scientific seminars on *in vitro* methods.

Every two years, SETOX organises the TOXCON conferences in Slovakia. This conference series has gained an international reputation and has become one of the most renowned, most highly appreciated conferences in central and eastern European countries, with the complimentary attribute of “having a family atmosphere”. In addition to its regular participants, many outstanding specialists from Austria, Estonia, Germany, Hungary, India, Iran, Israel, Italy, Poland, Spain, Sweden, Turkey and the USA have participated in TOXCON. It is rather encouraging that the number of participants has been steadily growing. One of the major achievements of SETOX was the organisation of the EUROTOX 2017 congress in Bratislava, Slovakia, which was attended by more than 1200 participants from 53 countries and included many sessions that were focused on *in vitro* toxicology. SETOX has its registered seat at the Slovak Academy of Sciences, which is a government-supported, highly ranked research institution.

SETOX’s board is formed mostly of scientists working at the Institute of Experimental Pharmacology and Toxicology (IEPT) at the Centre of Experimental Medicine (CEM) at the Slovak Academy of Sciences. IEPT is a GLP-certified organisation, and specialised laboratories at IEPT are conducting *in vivo*, as well as *in vitro* toxicity testing for regulatory and non-regulatory purposes. The IEPT joined the European Union Network of Laboratories for the Validation of Alternative Methods (EU NETVAL) in 2020, and is involved in the JRC project focusing on the validation of alternative methods addressing endocrine disrupting chemicals.

There is one private organisation in Slovakia with significant input in relation to the concept of Three Rs — MatTek *In Vitro* Life Science Laboratories, s.r.o. The company was established in 2009 in Bratislava, as a wholly owned subsidiary of the MatTek Corporation (Ashland, MA, USA) — a world-leading pioneer in the development and application of reconstituted human tissue models. The facility in Bratislava is very active in European validation studies and research projects leading to the development of new alternative methods. In addition to its production and research activities, it also provides training for scientists in the application of selected OECD test methods, and in general tissue and cell culture practice. MatTek *In Vitro* Life Science Laboratories is a corporate member and supporter, not only of SETOX, but also of the European Society for Toxicology *in Vitro* (ESTIV), the European Society for Alternatives to Animal Testing (EUSAAT) and the Italian Association of *In Vitro* Toxicology (CellTox).

Among academic institutions, several universities in Slovakia are involved in practical training and education in

some specific fields of Three Rs: the Medical University in Bratislava, the University of Agriculture in Nitra, the Technical University in Bratislava and the Veterinary University in Košice.

The Ministry of Agriculture and Rural Development of the Slovak Republic (MARD SR) established a national scientific network on alternative methods (the NOVS Committee) in 2007, to actively support Slovak representation in EURL ECVAM’s Network for Preliminary Assessment of Regulatory Relevance (PARERE). The membership of the national network includes representatives of the MARD SR, the State Veterinary and Food Administration (SVFA SR), the Ministry of the Environment, the Public Health Authority and individual members from the Slovak Academy of Sciences, Slovak universities and the private sector. Members of the NOVS Committee are regularly updated about EURL ECVAM activities, and contribute their scientific expertise and feedback on circulated documentation to the PARERE network. Some of the NOVS Committee experts also work on OECD Test Guideline expert panels. The SVFA SR at the MARD SR is the national contact point for the implementation of *Directive 2010/63/EU* on the protection of animals used for scientific purposes. It is also the Slovakian administrative authority involved with the laws on veterinary care and protection (*Act of the Slovak National Council No 39/2007 Coll.*), and on food products (*Act of the Slovak National Council No 152/1995 Coll.*). As the competent authority, it evaluates, regulates and approves proposals for animal experiments. It also approves and regulates animal breeding facilities, conducts inspections and ensures the compliance of breeders and suppliers, as well as the users of the animals, with national and the EU laws. Through its webpage, the SVFA SR provides information about new EU legislation related to animal protection. The SVFA SR is the expert guarantor of educational seminars and training courses in the areas of animal use and animal protection; introduction into the concept of the Three Rs is an element of the training.

SNP3Rs: The need for implementation of *Directive 2010/63/EU* on the protection of animals used for scientific purposes in Slovakia led to many discussions on how to cope effectively with the various aspects of this complex EU law. The idea of establishing a national Three Rs centre, that could conduct practical work and be an information resource on alternatives, had been under discussion among Slovak scientists, regulators and industry since 2015. The concept of a centre was presented again in 2016 at toxicology meetings in Slovakia, but it took another two years for the eventual launch of a national platform.

The platform was officially launched at the annual meeting of the Slovak Toxicology Society SETOX on 21 June 2018, at the TOXCON 2018 toxicology conference, under the name ‘The Slovak National Platform for 3Rs’

(SNP3Rs). SNP3Rs mirrors the activities of IVD-SETOX in the area of the Three Rs and communicates these activities to a broader audience outside the toxicology field. The current function of the SNP3Rs platform is to develop and encourage collaboration between individuals and organisations from various fields, in order to promote implementation of the Three Rs. It is proposed that the platform will further develop into a full Three Rs centre in the future.

At the national level, the activities of SNP3Rs are supported by the MARD SR and SETOX, as well as individual members from academia and governmental organisations involved in the implementation of the EU legislation on animal welfare. At the international level, SNP3Rs collaborates closely with the Czech 3Rs-Centre, located at the National Institute of Public Health (NIPH) in Prague. In 2020, SNP3Rs joined the European network of Three Rs centres, affiliated to the European Society for Alternatives to Animal Testing (EUSAAT).

The platform's representatives are actively involved in education, as well as in the development, validation and implementation of alternative methods into regulatory frameworks. Many of them are members of national and international committees and associations relevant to the Three Rs. SNP3Rs closely collaborates with the national contact point for implementing *Directive 2010/63* (i.e. the SVFA SR, which is affiliated with the MARD SR).

Another important partner and supporter of SNP3Rs is the Department of Food Safety and Nutrition, which is the national contact point for activities associated with the PARERE network, as defined in Article 47 of the *Directive 2010/63/EU*. Several SNP3Rs experts support the MARD SR and help with the processes required for the implementation of the regulations. Slovakia also has a national laboratory with expertise in validation studies, which is located at the Centre of Experimental Medicine of the Slovak Academy of Sciences and affiliated with the EU NETVAL network (at EURL ECVAM). Since its establishment, representatives from SNP3Rs participated in the scientific committee of the 2019 TOXCON congress and supported the organisation of a session focused on the implementation of the Three Rs in Slovakia. In 2021, SNP3Rs participated in the preparation of an OECD- and EFSA-supported virtual symposium, 'RegToxInVitro — Dissemination and implementation of the OECD *in vitro* and *in silico* methods applicable to the safety and risk assessment of the chemicals, food, and feed'.

Spain

There is presently great social, scientific, economical and industrial pressure to develop and implement methods capable of offering alternatives to animal experimentation, as well as supporting the *reduction* and *refinement* of animal use in preclinical research. In 1997, the Spanish network for

the development of Alternative Methods (REMA; www.buscaalternativas.com) was created to inform and promote alternative methods. Ethical committees at Spanish research institutions typically promote the Three Rs, but CMCiB is the only centre that has the promotion of the Three Rs concept as one of its goals.

CMCiB: The Comparative Medicine and Bioimage Centre of Catalonia (CMCiB) (www.cmcib.cat) is a research centre within the Germans Trias i Pujol Research Institute (IGTP; www.igtp.cat), which is dedicated to increasing scientific knowledge and promoting its transfer in order to improve the treatment, care and lives of patients. The IGTP is attached to one of the largest teaching hospitals in the Barcelona area, the Germans Trias i Pujol University Hospital, from the Autonomous University of Barcelona. The CMCiB, inaugurated in 2019, is a unique 4500 m² new multidisciplinary research and training facility, designed to meet the needs of comparative medical research. It is devoted to applying the Three Rs principles to promote the use of alternative methods in preclinical research. Studies being carried out at the centre include an extensive range of research projects, from preclinical studies using organoids, flies, fish, small and large animal models combined with bioimaging techniques, *in silico* and mathematical modelling, through to clinical research.

The CMCiB employs experts in the areas of animal welfare and husbandry, study design, bio-contention, surgery, diagnostic imaging and bioimaging, as well as computational analysis methods and mathematical modelling. The CMCiB works in collaboration with leading players in the private sector, to improve and refine research methods and drive forward technological innovation for the benefit of society. More information is available at cmcib@igtp.cat. CMCiB's Three Rs programme is funded by Fundació La Caixa (www.fundacionlacaixa.org). The zebrafish platform hosted at CMCiB is from ZeClinics (www.ZeClinics.com). Imaging equipment (MRI) is provided by Canon Medical Systems. Mathematical modelling is performed in collaboration with the Universitat Politècnica de Catalunya (UPC), in Barcelona (www.biocomsc.upc.edu).

Sweden

The Swedish definition of animal experiments is — and also has historically been — more extensive compared to that in the EU legislation. The Swedish definition is based on the purpose of the activities, and no animal suffering or risk of suffering is needed to define an animal experiment. This means that, in Sweden, animals used in, for example, behavioural studies or studies of housing conditions, animals euthanised to provide tissues or organs, as well as fish used in assessment studies, are all part of the national statistics on animals used in research.

During the 1970s, a general opinion against animal experiments emerged in Sweden. The Swedish Parliament raised the question of possibilities for increasing the control of research activities involving the use of animals, requesting an ethical review of projects before they were carried out, and highlighting the importance of considering alternative methods. These issues were investigated at length during the latter half of the 1970s. In 1978, Parliament decided that regional animal ethics committees should be instated, and ruled that animal experiments should only be permitted when alternative methods, that could provide equivalent data, did not exist. The regional animal ethics committees were instated in 1979, as was a central competent authority, The National Board for Laboratory Animals. At this point, the role of the committees was only advisory. It was proposed that the Chair and Vice-chair of each Swedish regional animal ethics committee should have legal training and experience as judges. Of the remaining members, half should be researchers, laboratory animal technicians or staff; the other half should be laypeople, with some representing animal welfare organisations.

In 1988, the ethical review of animal experiments was extended to cover, in principle, all uses of animals in research and not just the more invasive experiments. In 1998, the decisions of the regional animal ethics committees became legally binding, and an appeal function was installed. Since the beginning of the 1980s, the Swedish government has allocated an annual research grant to projects aimed at promoting the development of alternatives methods according to the Three Rs principles. In addition to the Swedish 3Rs Center, the Swedish Centre for Animal Welfare (SCAW) and the 3Rs Academy are important institutions working in the field.

Swedish 3Rs Center: In 2011, the Swedish government commissioned the Swedish Board of Agriculture, the Swedish Centre for Animal Welfare and the Swedish Research Council to investigate how work on alternative methods to research using animals should be conducted in Sweden. The investigation concluded that a competence centre for Three Rs issues should be established, to enable different stakeholders to meet and pursue work with the Three Rs. In 2014, the Swedish Parliament decided to establish a national competence centre for the Three Rs. Later the same year, the government commissioned the Swedish Board of Agriculture to initiate the formation of the ‘Swedish 3Rs Center’, which was inaugurated in November 2017.

Switzerland

Prior to the Swiss 3Rs Competence Centre (3RCC), the 3R-Foundation was created in 1987 through the joint efforts of the Parliamentary Group for Animal Experimentation Questions (a public body), Interpharma (the association of

Switzerland’s research-based pharmaceutical industry) and the Foundation for Animalfree Research (an animal protection organisation). These efforts were overseen by the Federal Department of Home Affairs. The aim of the 3R-Foundation was to promote alternative research methods to animal experimentation through grants for research projects, as well as to implement and promote the Three Rs principles. To meet communication needs in the field, the 3Rs Network was set up in parallel to the 3Rs-Foundation, and from 2013 to 2019 it focused on promoting the exchange of Three Rs-relevant information among researchers in Switzerland.

Although this set-up proved successful in advancing Three Rs research, there was a wish to better align research funding with education and services meeting the needs of researchers, higher education institutions (HEI) and civil society. This led to the creation of the 3RCC in March 2018, as a national coordinated effort to address the multiple facets of the Three Rs. The 3RCC is a non-profit association financed by the State Secretariat for Education, Research and Innovation (SERI), the Federal Food Safety and Veterinary Office (FSVO) and Interpharma. Its members constitute all higher educational institutions in Switzerland that conduct research involving animals, alongside with Switzerland’s largest animal welfare organisation, partners from industry and policymakers.

The 3RCC is a scientific centre of national importance that promotes the Three Rs concept in Switzerland and facilitates its implementation in the life sciences through funding of high-quality research, education, monitoring and communication. Since its inauguration, the 3RCC has funded 15 research projects covering all aspects of the Three Rs, has organised educational events, and contributed to increasing the visibility and understanding of the Three Rs principles in Switzerland.

United Kingdom

The UK has always been at the forefront of the Three Rs since the publication of *The Principles of Humane Experimental Technique* by Russell and Burch, in 1959.¹ However, for many years, the initiative lay with animal welfare and anti-vivisection organisations and, as a result, the Three Rs were not highly regarded among the research community, despite the pioneering work of respected bodies such as the Royal Society for the Prevention of Cruelty to Animals (RSPCA; <https://www.rspca.org.uk>) and the Fund for the Replacement of Animals in Medical Experiments (FRAME; <https://frame.org.uk>). Indeed, the violent activities of certain anti-vivisection organisations during the 1980s and 1990s led to an increasingly polarised divide, marked by defensiveness and lack of transparency from both sides. Despite these developments, the RSPCA and FRAME have continued to be very active in the Three Rs field to date. To improve this situation further, the National

Centre for the 3Rs (NC3Rs) was created, to encourage understanding in the research community of the scientific benefits of the application of the Three Rs.

NC3Rs: The National Centre for the 3Rs (NC3Rs; <https://www.nc3rs.org.uk>) was launched in 2004 in response to a UK Government recommendation to establish a national centre to increase the focus on the Three Rs. The NC3Rs Mission⁴⁷ is pioneering better science to:

- support the discovery and adoption of predictive, reproducible and cost-effective alternatives to the use of animals.
- improve standards where animal use is necessary, optimising model selection and study design and minimising suffering as far as possible.
- promote the importance of the Three Rs across the scientific community, nationally and internationally, by providing training and embedding the Three Rs in policy, practice and regulations.

It collaborates with scientists and organisations from across the life sciences sector, nationally and internationally, including universities, industry, other research funders and regulatory authorities. These partnerships are essential for both the development of new Three Rs approaches and their implementation in practice. In the 17 years since the NC3Rs was launched, it has led a systematic and comprehensive transformation in the level of engagement, activity, acceptance and use of Three Rs approaches, which in turn has delivered scientific, ethical and economic benefits.

Starting from a low baseline, it has developed into one of the largest Three Rs bodies in the world, with 33 full-time staff and an annual budget of over £10 million. The success of the NC3Rs has been dependent on its science-focused strategy, which has concentrated on:

- raising awareness of the importance of the Three Rs and demonstrating their scientific validity and benefits (i.e. making the Three Rs and the NC3Rs credible);
- building a national and international reputation as a trusted partner to engage a wide range of scientific stakeholders;
- expanding work beyond research funding to influencing national and international policy, practice and regulations;
- introducing innovative programmes such as CRACK IT;
- maximising the impact of the NC3Rs by analysing and addressing the barriers to the spread of Three Rs thinking and approaches in the scientific community; and
- making the Three Rs standard practice globally and in all sectors.

The highlights of the NC3Rs include: research and innovation funding (e.g. the CRACK IT programme⁴⁸ and other research grants); training (e.g. PhD studentships and training fellowships); changing policy, practice and regulations (via specific programmes on experimental design and reporting, animal welfare, toxicology and safety sciences); and communication and dissemination (through publications and online resources).

Ukraine

Three Rs-related initiatives are very new in the Ukraine.

Ukrainian 3Rs Center: At the beginning of 2020, an initiative by a group of scientists working in the fields of *in vitro* and *in silico* toxicology led to the creation of the Ukrainian 3Rs Center (UN3RC). This initial group invited further scientists working in the area of *in vivo* toxicology (with experience in developmental neurotoxicity (DNT), developmental and reproductive toxicity (DART), sub-chronic and chronic toxicity) to become members. The members of the UN3RC strongly believe that only through such collaborative efforts can an effective way be found to use alternative methods such as *in vitro* and *in silico* models, which relate to the *replacement* principle of the Three Rs concept. The UN3RC is aware that, in some studies, animal experiments are still often used, and that *replacement* methods are far from being fully available for implementation (such as in the assessment of DNT, DART, chronic toxicity, etc.). Consequently, methods that support the Three Rs principles of *reduction* and *refinement* have to be promoted and implemented for the time being. In this regard, a major aim of the UN3RC is to educate people currently undertaking animal experiments about the availability of Three Rs alternative methods, to bridge the gap between *in vivo* and *in vitro/in silico* fields and generally raise awareness on the importance of the Three Rs concept.

Summary

This first article of the series describes the rise of European Three Rs centres and platforms. Milestones in the history of the development of the Three Rs centres and platforms were highlighted chronologically, showing the influence and importance of pan-European, but also of national, legislation and support. The institutions that contributed to this article summarised the developments of the Three Rs field in their own countries, as well as their individual stories. This narrative impressively underlines, on the one hand, the national and regional differences, and on the other, the common challenges associated with implementing the Three Rs principles in daily scientific work.

The histories of the development of the various Three Rs institutes are very diverse, with the founding and development work taking place under a range of very different









conditions. This shows that many paths are possible for the successful creation and running of Three Rs institutions, and this tenet can serve as an inspiration to other countries as they undertake their Three Rs journey.

The next articles in this series will focus on the current status of the Three Rs centres and platforms, their financial and organisational structures, their focus on specific Three Rs topics and projects, tools to advance the field, and future projects and plans. These contributions from the Three Rs centres and platforms emphasise their immensely important role and their potential to inspire scientists and enhance biomedical research with their Three Rs-based approaches. In addition, they also reveal the anchor points to intensify networking, learn from each other, and facilitate the contact of interested readers with the Three Rs centres and platforms. These articles will also highlight the future needs and opportunities for improvement of the Three Rs institutions, so that they are able to optimally fulfil their goals and the expectations placed upon them.

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Appendix. The acronyms and abbreviations mentioned in the article.

Acronym/abbreviation	Explanation
3RCC	Swiss 3Rs Competence Centre
AFTF	<i>Arbeitskreis für die Förderung von tierversuchsfreier Forschung</i> (Working Group for the Promotion of Animal-Free Research; Austria)
AOP	Adverse Outcome Pathway
BB3R	Berlin-Brandenburg Research Platform
Bf3R	German Centre for the Protection of Laboratory Animals
BfR	German Federal Institute for Risk Assessment
BGA	Federal Health Office (Germany)
BgVV	Federal Institute for Health Protection of Consumers and Veterinary Medicine (Germany)
BMBF	Federal Ministry of Education and Research (Germany)
BPAM	Belgian Platform for Alternative Methods
CAAT	Center for Alternatives to Animal Testing (USA)
CAD	Coordination Point for Alternatives to Animal Testing (Netherlands)
CERST-NRW	Centre for Alternatives to Animal Testing funded by the Ministry for Culture and Science of the State of North-Rhine Westphalia (Germany)
Charité 3 ^R	Faculty-overarching structure to foster the implementation of the Three Rs at Charité–Universitätsmedizin Berlin
CIMH	Central Institute of Mental Health (Mannheim, Germany)
CLASA	Czech Laboratory Animal Science Association
CMA	National Centre for Alternative Methods to Toxicity Assessment (Poland)
CMCiB	Comparative Medicine and Bioimage Centre of Catalonia (Spain)
CoC Network	Culture of care network
CONGENTO	Consortium for Genetically Tractable Organisms
CTFA	US Cosmetic, Toiletries, and Fragrance Association
Czecopa	Three Rs Czech Consensus Platform for Alternatives
DACOPA	Danish Consensus Platform for Alternatives to Animal Experiments
DFG	German Research Foundation
DK-EPA	Danish Environmental Protection Agency
ECHA	European Chemicals Agency
ecopa	European Consensus-Platform for Alternatives
EFSA	European Food and Safety Authority
ERGATT	European Research Group for Alternatives in Toxicity Testing
ESAC	EURL ECVAM Scientific Advisory Committee
ESTIV	European Society for Toxicology <i>In Vitro</i>
ETPLAS	Education and Training Platform for Laboratory Animal Science
EU3Rnet	European Network of 3R Centres
EURL ECVAM	European Reference Laboratory for Alternatives to Animal Testing
EUROTOX	European Societies of Toxicology
EUSAAT	European Society for Alternatives to Animal Testing
FELASA	Federation of European Laboratory Animal Science Associations

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Acronym/abbreviation	Explanation
FFvFF	<i>Fonds für versuchstierfreie Forschung</i> (Animal-free Research Fund)
FRAME	Fund for the Replacement of Animals in Medical Experiments (UK)
FSVO	Swiss federal food safety and veterinary office
FU Berlin	Freie Universität Berlin (Germany)
HPRA	Irish health products regulatory authority
IC-3Rs	Innovation Centre 3Rs (Vrije Universiteit Brussel, Belgium)
ICAR3R	Interdisciplinary centre for 3Rs in animal research at the Justus Liebig University (JLU), in Giessen (Germany)
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods (USA)
ICLAS	International Council for Laboratory Animals Science (Poland)
Icopa	Irish consensus platform for alternatives
IEPT	Institute of Experimental Pharmacology and Toxicology (Slovak Republic)
ISPCA	Irish Society for the Prevention of Cruelty to Animals
IUF	<i>Leibniz Institut für umweltmedizinische Forschung</i> (Leibniz Research Institute for Environmental Medicine), in Düsseldorf (Germany)
IVTD	<i>In Vitro Toxicology and Dermato-Cosmetology</i> (at Brussels Health Campus, Belgium)
JRC	European Commission Joint Research Centre
MARD SR	Ministry of Agriculture and Rural Development (Slovak Republic)
MEGAT	<i>Mitteeuropäische Gesellschaft für Alternativmethoden zu Tierversuche</i> (Middle European Society for Alternatives to Animal Experiments)
MUI animalFree Research Cluster	Medizinische Universität Innsbruck animalFree Research Cluster
NAM	New approach methodology
NC3Rs	The National Centre for the 3Rs (UK)
NCA	National Centre for Alternatives to Animal Testing (Netherlands)
NCad	Netherlands National Committee for the protection of animals used for scientific purposes
NETVAL	European Union Network of Laboratories for the Validation of Alternative Methods
NIOM	Nofer Institute of Occupational Medicine in Lodz (Poland)
Norecopa	Norwegian Consensus-Platform for Alternatives
NOVS Committee	National scientific network on alternative methods (Slovak Republic)
OECD	Organisation for Economic Co-operation and Development
PARC	Partnership for the Assessment of Risk from Chemicals (H2020 project)
PARERE	EURL ECVAM's Network for Preliminary Assessment of Regulatory Relevance
PETA	People for the Ethical Treatment of Animals
Polcopa	Polish Consensus-Platform for Alternatives
R2N	Initiative 'Replace and Reduce from Lower Saxony' (Germany)
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
REMA	Spanish network for the development of Alternative Methods
RepRefRed Society	<i>Gesellschaft zur Förderung von Alternativen Biomodellen</i> (co-operation of the medical universities of Graz, Innsbruck and Vienna, which aims to promote the Three Rs; Austria)
RIVM	National Institute for Public Health and the Environment (Netherlands)
ROCAM	Romanian Centre for Alternative Test Methods
RSPCA	Royal Society for the Prevention of Cruelty to Animals (UK)
SAS	Slovak Academy of Sciences
SCAW	Swedish Centre for Animal Welfare
SCCT	Scandinavian Society for Cell Toxicology
SETOX	Slovak Toxicology Society
SNP3Rs	Slovak National Platform for 3Rs
SPCAL	<i>Sociedade Portuguesa de Ciência em Animais de Laboratório</i> (Portuguese Society of Laboratory Animal Science)
SPEdH	<i>Sociedade Portuguesa para a Educação Humanitária</i> (Portuguese Society for Humane Education)

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Acronym/abbreviation	Explanation
SVFA SR	State Veterinary and Food Administration (Slovak Republic)
t ⁴	Transatlantic think tank for toxicology
TARCforce 3R	Research Center for Animal Welfare and Laboratory Animal Science (Germany)
TCD	Trinity College Dublin
THM	Technical University of Central Hessen (Germany)
TPI	Transition Programme for Innovation (Netherlands)
TU Berlin	Technische Universität Berlin (Germany)
UFAW	Universities Federation for Animal Welfare (UK)
UN3RC	Ukrainian 3Rs Center
VUB	Vrije Universiteit Brussel (Belgium)
VZET	<i>Virtuelles Zentrum für Ersatz- und Ergänzungsmethoden zum Tierversuch</i> (Virtual Centre for Alternative and Complementary Methods to Animal Testing), University of Veterinary Medicine, Hannover (Germany)
ZEBET	<i>Zentralstelle zur Erfassung und Bewertung von Ersatz- und Ergänzungsmethoden zum Tierversuch</i> (Central Office for the Registration and Evaluation of Alternative and Complementary Methods to Animal Experiments), Germany
ZET	<i>Zentrum für Ersatz- und Ergänzungsmethoden zu Tierversuchen</i> (Centre for Replacement and Complementary Methods to Animal Experiments), Germany