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The Current Status and Work of Three Rs Centres and Platforms in Europe*

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Abstract

The adoption of *Directive 2010/63/EU* on the protection of animals used for scientific purposes has given a major push to the formation of Three Rs initiatives in the form of centres and platforms. These centres and platforms are dedicated to the so-called Three Rs, which are the Replacement, Reduction and Refinement of animal use in experiments. *ATLA*'s 50th Anniversary year has seen the publication of two articles on European Three Rs centres and platforms. The first of these was about the progressive rise in their numbers and about their founding history; this second part focuses on their current status and activities. This article takes a closer look at their financial and organisational structures, describes their Three Rs focus and core activities (dissemination, education, implementation, scientific quality/translatability, ethics), and presents their areas of responsibility and projects in detail. This overview of the work and diverse structures of the Three Rs centres and platforms is not only intended to bring them closer to the reader, but also to provide role models and show examples of how such Three Rs centres and platforms could be made sustainable. The Three Rs centres and platforms are very important focal points and play an immense role as facilitators of *Directive 2010/63/EU* 'on the ground' in their respective countries. They are also invaluable for the wide dissemination of information and for promoting the implementation of the Three Rs in general.

Keywords

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

Introduction

In the first article in the series about Three Rs centres and platforms, milestones in the history of the Three Rs were described and the most important events in the field were summarised for the respective European countries.[1] This illustrated key developments in the Three Rs field, not only in Europe as a whole, but also in each of the individual countries. In this second article, the national Three Rs institutes (see Table 1) that contributed to the first article have provided detailed information on their current status and activities. As before, in view of the wide diversity of such organisations, each centre was allowed to

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Table 1. Information about the 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/
Einstein Center 3R	Germany	Centre	https://www.ec3r.org/
ICAR3R–3R Centre JLU Giessen	Germany	Centre	https://www.uni-giessen.de/fbz/zentren/icar3r
Leibniz Alternatives at IUF–Leibniz 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 Center Rhine Neckar	Germany	Centre	https://en.3r-rn.de/
VZET Hannover	Germany	Three Rs network	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	Centre	https://wwwen.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 Center 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
Swiss 3R Competence Centre (3RCC)	Switzerland	Centre	https://swiss3rcc.org/
National Centre for the 3Rs–NC3Rs	UK	Centre	https://www.nc3rs.org.uk/
The Ukrainian 3Rs Center	Ukraine	Centre	https://nuft.edu.ua/en/nnixt/ktzpkp/

contribute in its own way, with minimal editorial control exerted over the length and content of the individual contributions.[1] Since the Three Rs field and the establishment of Three Rs centres and platforms is very dynamic, we do not claim to present an exhaustive overview. Rather, we have attempted to provide a representative snapshot of the current situation.

All Three Rs centres and platforms are committed to the common goal of pursuing the Three Rs principles in their research and education. The ground-breaking book by William Russell and Rex Burch, *The Principles of Humane Experimental Technique*[2] (http://altweb.jhsph.edu/pubs/books/humane_exp/het-toc), published in 1959, set the foundation for the Three

Rs. Even though each Three Rs organisation is committed to the Three Rs (Replacement, Reduction and Refinement of animal use in experiments), each has its own focus on one or more specific 'R'. In addition, each Three Rs centre and platform has its unique financial and organisational structure, and its own ways of disseminating information and providing education to help implement the Three Rs, including putting the issues of scientific quality and ethics into practice. This wide diversity has led to the fact that, in reality, there is no real standard definition of a 'Three Rs centre'. In comparison to Three Rs centres, Three Rs platforms tend to have looser structures.

Nonetheless, there are major common tasks to be tackled by these platforms, such as supporting implementation of the Three Rs in terms of *Directive 2010/63/EU* on the protection of animals used for scientific purposes,[3] the EU legislation on REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals, *EC 1907/2006*),[4] and the animal testing and marketing ban on cosmetics. In addition, it is a great challenge to make the Three Rs more widely known and established in basic science, in order to come closer to achieving the goal of better biomedical science. This includes focusing on all five areas that have clearly been identified as priorities by the centres and platforms, namely: dissemination; education; implementation; scientific quality/translatability; and ethics. To improve knowledge exchange and co-operation, many European Three Rs centres and platforms have organised themselves within EU3Rnet.[5,6] In the following sections, the individual Three Rs centres and platforms have provided insights into their current status and work.

Financial backing and support of the Three Rs centres and platforms

As shown previously,[1,7] the Three Rs centres and platforms have a variety of founding histories, having been either created by governmental bodies or initiated by individuals or smaller groups through their own commitment to the cause. In the same way, the funding needed by the Three Rs centres and platforms is obtained from a wide range of sources; this is summarised in Figure 1. Several sources of financial backing are used, and individual Three Rs centres and platforms may receive support from several different channels, which have been categorised accordingly. Of the Three Rs centres and platforms surveyed, 71% are embedded in academic institutions, 43% receive governmental/public support, and 39% receive no monetary support at all. Industry sponsors are partners of 18% of the Three Rs centres and platforms, whereas 11% receive financial grants from philanthropists, 7% from animal welfare organisations, and 4% of the centres/platforms are supported by membership fees. To further illustrate the diversity of the different financial models, several examples are presented below:

— The *Austrian RepRefRed Society* is financed by the Austrian Federal Ministry of Education, Science and

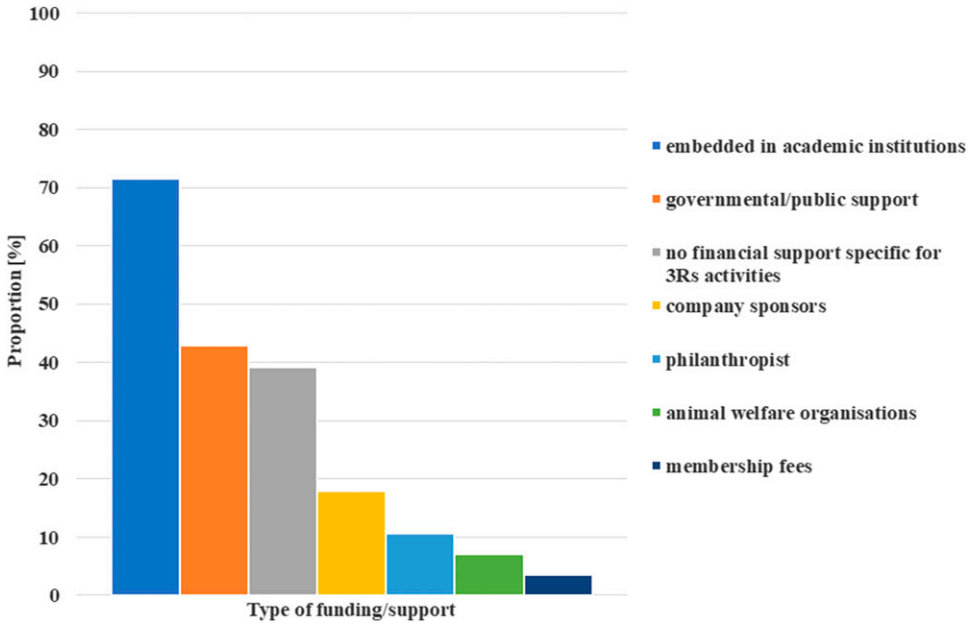

Figure 1. The diverse financial backing and support of the Three Rs centres and platforms. The graph shows the proportion (%) of the featured Three Rs centres and platforms (total n = 29) that mentioned the different types of funding and support (multiple sources of funding are possible for each centre/platform).

Research for establishing the Austrian 3R Centre, and by membership fees from private persons, companies and academic institutions.

— The *Belgian IC-3Rs* receives financial support from corporates for the Three Rs symposia it organises, and from the Brussels Region and Flanders governmental initiatives for funding IC-3Rs projects, such as Re-Place and its research group IVTD. Recently, philanthropist Mireille Aerens bequeathed her fortune to the Vrije Universiteit Brussel (VUB), with the clear wish to support IC-3Rs and the research group IVTD. IC-3Rs can now further expand and IVTD can provide more researchers with the opportunity to do Three Rs-based research and develop novel human-based methodology without the use of experimental animals.
— The *3Rs Centre Czech Republic* is a public establishment seated within a governmental institution, i.e. the National Institute of Public Health in Prague, and co-operates with a number of experts in the Czech Republic. It has received limited financial support from the Ministry of Agriculture, which is responsible for the implementation of *Directive 63/2010/EU* in the Czech Republic. Its budget can be used for the development and validation of new alternative methods, the dissemination of information, and for advisory services. Additional financial resources are obtained via grants for specific research projects, but the undertaking of routine activities is financed from the budget of the host institution. The *3Rs Centre Czech Republic* is open to all interested researchers, members of animal welfare bodies, manufacturers of medical devices, cosmetics and other consumer products, and academics and students, who together serve to create a national network.
— The *Danish 3R-Center* is financed by the Danish government, three pharmaceutical companies and two animal welfare organisations.

Some examples of funding strategies in Germany

Analysis of the various Three Rs centres and platforms in Germany reveals a very diverse picture in terms of financial support:

— The *BB3R* founding members received an initial funding for 3–4 years from the Federal Ministry of Education and Research. Since then, the working group leaders have financed their projects mainly from their own funds, or those of their institutions. For example, the Freie Universität Berlin has supported their scientists substantially by means of a special grant.
— *CAAT-Europe* is part of the University of Konstanz. It is funded by corporate sponsors, organisations and philanthropic donors. In addition, it tries to raise money by participating in competitive grants at federal state, national and EU level.
— Universitätsmedizin Berlin *Charité 3^R* is part of Charité–Universitätsmedizin Berlin the university hospital of Berlin and the joint medical faculty of Freie Universität Berlin (FU Berlin), and Humboldt Universität zu Berlin (HU Berlin). It was funded by Charité from 2018–2022 with up to €2 million per year (governmental funding from the State of Berlin); a continuation of the funding is intended. Furthermore, in accordance with the Berlin Higher Education Agreement 2018–2022, Charité 3R, together with BB3R and the Berlin partners, advocated for an Einstein 3R Center (*EC3R*) (https://www.ec3r.org/de/).[8,9] In December 2019, the Senate provided the Einstein Foundation with a total of €5.3 million for 2020–2026 and, together, the FU Berlin, HU Berlin, TU Berlin, MDC, RKI, BfR, and Charité successfully initiated the Einstein Center 3R, which finally became active in July 2021.
— The *ICAR3R* is part of the Justus-Liebig-University Giessen and financed by university funds and the Hessian Ministry for the Environment, Climate Protection, Agriculture and Consumer Protection. Furthermore, certain projects are financed by third parties, such as: 'Transparency in the handling of laboratory animals (the LIVE3R platform)', which is funded by the KOGGE Foundation; 'Culture of Care', funded by the SET Foundation (Foundation for the promotion of alternate and complementary methods to reduce animal experiments); 'Quality of life of dogs and cats with chronic progressive diseases', funded by Boehringer Ingelheim; and 'Medical training', funded by GKF–Gesellschaft zur Förderung Kynologischer Forschung e.V.
— *Leibniz Alternatives* is part of the IUF–Leibniz Research Institute for Environmental Medicine and, as such, it is embedded in an academic institute. All projects so far have been funded by third-parties, mostly through public funds (e.g. the CERST-NRW (*Center for Alternatives to Animal Testing*), funded by the Ministry for Culture and Science of the State of North-Rhine Westphalia, the German Research Foundation (DFG), and the EU Commission Horizon 2020 funding programme), yet it also collaborates on projects with industrial partners (e.g. Cefic-LRI, Clariant, BASF, Syngenta) and regulators (EFSA, US-NTP, DK-EPA). In addition, Leibniz Alternatives also offers assay development, substance testing and consultancy services.
— *TARCforce3R* is financed by the University Medical Center and receives project-specific funding by application for research grants.

— The *3R-Center Rhine-Neckar* is up to 70% financed by the Ministry for Science, Research and Art Baden-Wurttemberg, Germany, and up to 30% financed by the board of the Institute of Psychopharmacology at the CIMH, for a 5-year duration. Following this period, the CIMH alone will aim to secure funding for the centre.
— *VZET* is an association of the scientists working in the Three Rs field at the University of Veterinary Medicine Hannover (TiHo), Germany. While it has no specific funding, the scientific projects of the scientists involved are funded by the public sector — for example, by the German Research Foundation (DFG), the German Ministry of Education and Research (BMBF), and the Ministry of Science and Culture of Lower Saxony (MWK) (e.g. in the R2N network). Additionally, international young scientists are frequently involved via funding from the German Academic Exchange Service (DAAD) or the Alexander von Humboldt Foundation.

Further examples of public sector and government funding

Other Three Rs centres and platforms throughout Europe that are financed by the public sector and governments include NCad, Norecopa, the Swedish 3Rs Center, the Swiss 3RCC and the NC3Rs in the UK.

Norecopa receives core funding — which covers expenses related to the Secretary and the running costs — from two ministries. It also receives income from its members (institutions and individuals), and from animal welfare organisations, legacies and the Norwegian Research Council on a case-by-case basis.

The *3RCC* receives funding from federal funders (the Swiss State Secretariat for Education, Research and Innovation (SERI) and the Swiss Federal Food Safety and Veterinary Office (FSVO)), as well as from sponsorship by the Swiss association of research-based pharmaceutical companies (Interpharma). According to the Federal Act on the Promotion of Research and Innovation (RIPA) Article 15, the federal funds are provided by the member institutions as in-kind contributions. The 3RCC members include 11 universities and higher education institutions in Switzerland: Ecole Polytechnique Fédérale de Lausanne (EPFL), the Federal Institute of Technology Zurich (ETH Zurich), Università della Svizzera Italiana (USI), the University of Applied Sciences and Arts Northwestern Switzerland (FHNW), the University of Basel, the University of Bern, the University of Fribourg, the University of Geneva, the University of Lausanne, the University of Zurich, and the Zurich University of Applied Sciences (ZHAW). Moreover, Interpharma, the FSVO, and the Swiss Animal Protection (SAP) organisation are also members of 3RCC. In addition, SERI and the Swiss Federal Office of Public Health (FOPH), act as observer members. The 2021 financial forecast is CHF 4.35 million, of which CHF 2.17 million is provided by the funding bodies (SERI, FSVO and Interpharma) and CHF 2.19 million as the projected in-kind contribution by the partners.

The *Swedish 3Rs Center* and the UK's *NC3Rs* are financed by the Swedish and British governments, respectively. The NC3Rs receives approximately £10 million per annum from core funders (the UK's Medical Research Council and the Biotechnology and Biological Sciences Research Council), and approximately £1 million from other sources (e.g. industry or charities for grant co-funding, specific activities and posts). In addition, there is a large in-kind contribution from industry partners in the CRACK IT programme.

Centres that are embedded within academic institutions

Some Three Rs centres are embedded within academic institutions and receive financial support from them. These include the 3Rs Centre Utrecht (the Netherlands), the 3R Centre at i3S (Portugal) and the CMCiB (Spain).

The *3Rs-Centre in Utrecht* is financed by Utrecht University and University Medical Hospital Utrecht through a collaboration with the Animal Welfare Body and the Faculty of Veterinary Medicine, whereas the *3R Centre at i3S* is part of the academic research institution i3S, currently (until 2022) benefitting from Horizon 2020 funding through the REMODEL project. The Three Rs programme at *CMCiB* is financed for a five-year period by the private institution Fundación La Caixa.

Several other Three Rs centres and platforms, such as *Trinity College Dublin*, do not receive any Three Rs-specific funding. The salaries of the members of the Trinity College Comparative Medicine Unit (TCD-CMU) that form the incipient *icopa* are paid by their current employers and their jobs are not specifically dedicated to the Three Rs. This represents more of a voluntary activity for all of them. However, within the Laboratory for Biological Characterisation of Advanced Materials at Trinity (LBCAM), the Three Rs regulatory science research is, and has been, supported by EU funding, e.g. EU Framework Programme 7 and Horizon 2020.

Centro 3R in Italy, the National Centre for Alternative Methods to Toxicity Assessment (*CMA*) in Poland, the *Ukrainian 3Rs Center*, the *Luxembourg 3Rs Platform* or the *SNP3Rs* in the Slovak Republic, do not currently receive any Three Rs-specific monetary support. The CMA tries to encourage collaboration between various stakeholders, mainly from the cosmetics and pharmaceutical industries, which is one of the goals of the new Horizon 2020 project entitled 'Twinning for Excellence in Alternative Toxicity Assessment Methods' (TWINALT; twinalt.com), conducted in partnership

with three outstanding research partners specialists in alternative methods from Belgium (the Vrije Universiteit Brussel; VUB), Norway (Norwegian Institute for Air Research; NILU) and Italy (Università degli Studi di Milano; UMIL). This topic is expanded further in the section on *Detailed activities of the Three Rs centres and platforms*.

The Luxembourg 3Rs Platform plans to ideally involve different stakeholders (universities/academia, public research centres, government, national funding agency, animal welfare associations) in their future activities. Similarly, the Slovak platform SNP3Rs, supported by SETOX and MARD SR, is open to all parties interested in Three Rs research (researchers, animal welfare bodies, industry, academics and students). It operates on a *pro-bono* basis, i.e. voluntary work in the scope of the expertise of the individual participants.

Conclusions about financial backing and support

In summary, there is already significant financial support from the state in some countries — but it would be desirable for the Three Rs centres and platforms that have emerged from personal initiatives to receive financial support from their governments, in order to ensure sustainability of their efforts in the Three Rs field.

Organisational structures of Three Rs centres and platforms

In this section, we introduce the organisational structures of the Three Rs centres and platforms. These can vary distinctly, from very hierarchical structures, with a board and several committees, through to very flat hierarchies with only a few people in charge.

Austria

The *Austrian RepRefRed Society* consists of the board (six members) and other paying members. Members of the board are not paid for their work, except for two assistants (who are also part of the board). The assistants undertake organisational tasks (e.g. public relations, marketing, event management, etc.) and their costs are covered by the RepRefRed Society on a project basis.

Belgium

The structure of the Belgian *IC-3Rs* consists of a board with seven members (chair and co-chair, a Steering Scientific Committee with national and international experts on the Three Rs), about 10 active young volunteer scientists within the IVTD, and two people in IVTD's secretariat helping with administrative matters. The Chair and Steering Committee members receive no payment; the Co-chair is employed by the university as head of IVTD (on a fixed-term contract). The young scientists are employed by the university on project-based contracts, with grants from different sources (partly also from the IC-3Rs).

Czech Republic

The network of the *3Rs Centre Czech Republic* comprises around 100 participants with various fields of expertise, but it is not strictly organised in a hierarchical structure.

Denmark

The *Danish 3R-Center* consists of a chairperson and six members, all with high levels of professional expertise and experience in at least one of the Three Rs. The seven board members also constitute The National Committee for the Protection of Animals Used for Scientific Purposes. The board members receive an annual fee for their work. The 3R-Center (and thereby the National Committee) also has a secretariat consisting of four people employed by the Danish Veterinary and Food Administration.

Germany

The Giessen 3R Center of the Justus Liebig University Giessen *ICAR3R* (Interdisciplinary Center for 3Rs in Animal Research) was formed out of an initiative to strengthen science in Hessen and establish the Central Hesse Research Campus. The interdisciplinary approach of the centre is already reflected in its basic structure, with the centre's management being the responsibility of two complementary Three Rs professorships — one in the Faculty of Veterinary Medicine and the other in the Faculty of Medicine. The Executive Director is Univ. Prof. Dr med. vet. Stephanie Krämer, who holds the Professorship for Laboratory Animal Science and Animal Protection at the Faculty of Veterinary Medicine at Justus-Liebig-University; the Deputy Director is Prof. Dr Peter Jedlicka, who holds the Professorship for Computer-based Modelling in the field of 3R Animal Protection at the Faculty of Medicine at Justus-Liebig-University. These two positions form the 'roof' of the centre, which is supported by a coordinator and a programme coordinator. The members of the centre form the board of directors, which is its decision-making body. These members range from scientific staff, doctoral students and technical staff, through to student representatives. The directorate meets at least once a year. Currently, there are 11 co-workers in the ICAR3R, either permanently

employed or holding project-based contracts. The Executive Director and Deputy Director hold permanent contracts.

CAAT-Europe is a joint venture between Johns Hopkins Bloomberg School of Public Health, USA, and the University of Konstanz, Germany, directed by Prof. Dr Marcel Leist and Prof. Dr Thomas Hartung. Besides the two directors, there are four (part-time) staff members, including a CEO, and their types of contract vary.

Another centre in Germany — the *3R-Center Rhine-Neckar* — is coordinated by Dr Marcus Meinhardt. Together with a scientific team comprised of Prof. Rainer Spanagel, Prof. Sabine Chourbaji, Dr Bettina Kränzlin, Dr Stefanie Kirschner and Dr Matthias Braun, Dr Meinhardt plans and conducts all activities of the centre. These six coworkers have either project-based or permanent contracts at their respective universities.

The *R2N* includes a total of 14 network members covering different topics: four members focus on alternatives in basic research, four work on alternatives in the regulatory framework and safety testing, three cover tissue and cell biobanking, two focus on ethical aspects, and one member performs systematic reviews. These individuals are located within seven different institutions in Lower Saxony. The consortium is supported by the government of Lower Saxony and is preparing for subsequent funding from 2022 onwards.

Leibniz Alternatives is a public–private partnership platform located at the IUF, which is headed by Prof. Dr Jean Krutmann (Scientific Director) and Dr Alexander Beaucamp (Financial Director). Prof. Dr Ellen Fritsche is head of Leibniz Alternatives and holds the W2 Professorship for Environmental Toxicology at the Faculty of Medicine at the Heinrich-Heine-University, Dusseldorf. Dr Julia Tigges is the operational manager of this Three Rs platform, which is financed exclusively by third-party funded projects.

Also in Germany, the Three Rs centre *TARCforce3R* is independent, but incorporated in the Translational Animal Research Center of the University Medical Centre Mainz. Therefore, there is a direct connection between the animal facility and TARCforce3R. There is a head of the Three Rs centre, two postdoctoral researchers and two PhD students. The head has a permanent contract, while the postdocs and PhD students hold project-based contracts.

The *BB3R* has 11 founding members, plus three junior professorships and nine associated members. Prof. Kleuser and Prof. Thöne-Reineke are BB3R spokespersons, while Dr Kral is the coordinator. All members meet on a regular basis to discuss the ongoing projects and future steps. Scientists working in the field of the Three Rs can apply for affiliation to the centre. A Scientific Advisory Board, whose members come from industry, academia and associations in Germany, serves to ensure consistent quality. PhD students working in one of the BB3R-associated groups can apply for the graduate programme. All BB3R members are responsible for funding their own research and their own employees. FU Berlin/BB3R interacts with other Three Rs centres, such as TARCforce3R in Mainz. Through, for example, FU Berlin-UZH Joint Seed Funding, there is research co-operation with the newly founded 3R Competence Center at the University of Zurich.

Charité 3^R is headed by a council of six spokespersons, representing different scientific backgrounds, career stages and the Charité animal welfare body. The council of spokespersons is elected by the members' assembly of approximately 150 Charité scientists, who are committed to a progressive Three Rs engagement. Operative tasks are conducted by a five-member office team. Charité 3^R is advised by two boards: an international Scientific Advisory Board and an additional local Berlin Advisory Board.

The Einstein Center 3R (*EC3R*) is a network of all relevant biomedical research institutions in Berlin.[9,10] The centre is headed by its spokesperson, Prof. Hippenstiel, and co-spokespersons, Prof. Thöne-Reineke and Prof. Kurreck. The activities of EC3R are supervised by a steering committee and an international scientific board. The EC3R research network comprises six scientific and two cross-sectional projects. Further activities include the education of young scientists in Three Rs approaches and fact-based communication.

In case of *VZET*, another Germany-based network, the board coordinates Three Rs activities, updates the online presence on ongoing projects and publications, and organises scientific symposia, planned in a two-year cycle. The VZET has 31 members (as of May 2022), and they are all employees of the University of Veterinary Medicine Hannover (TiHo), Germany. In this context, it is important to mention that, since 2018, the TiHo has implemented a Junior Professorship on alternatives/complementary methods to animal experiments (currently held by Prof. Bettina Seeger).

Ireland

At *Trinity College Dublin (TCD)* in Ireland, two institutes are mainly responsible for Three Rs activities. The TCD-Comparative Medicine Unit (CMU) is organised to run a laboratory animal programme at TCD, with technicians working closely with the animals, an administration section to deal with regulatory and compliance issues, and a veterinary team that comprises three members from the TCD-CMU part of the Three Rs initiative (Viola Galligioni, Daniel Ruiz Pérez and José M. Sánchez Morgado). As well as these three veterinarians, TCD-CMU has three technicians specifically involved in the work. All members of TCD-CMU are employed by TCD on a permanent basis.

The TCD Laboratory for Biological Characterisation of Advanced Materials (LBCAM) team is structured into three

thematic areas: regulatory science; biomedical research; and preclinical screening. Across these three thematic areas, the lead manager (Adriele Prina-Mello), the lead scientist (Dania Movia) and the multidisciplinary research team are building in the critical capacity to meet the Three Rs requirements of *reduction* and *replacement*. The TCD-LBCAM consists of five research staff members working on the Three Rs. With the exception of the lead manager, the members of the Trinity College LBCAM team are employed on project-based contracts.

Italy

Within the *Centro 3R* platform in Italy, each university is an independent operating unit (OU). Each OU is led by five or six elected representatives, and the representatives from each OU form the Didactics Scientific Counsel (CSD, 'Consiglio Scientifico Didattico'), with an elected Director and a nominated vice-Director. The CSD holds three or four meetings a year to discuss initiatives. There is no financial support for the Centro 3R platform.

Luxembourg

The *Luxembourg 3Rs Platform* is under development and is based on the work of 10–20 individuals. The University of Luxembourg, and the Luxembourg Institute of Science and Technology (LIST) and Luxembourg Institute of Health (LIH) research centres, employ permanent as well as temporary staff, who contribute to the platform's activities in addition to their normal work.

Netherlands

The *3Rs-Centre of the Utrecht University and University Medical Centre Utrecht* in the Netherlands is embedded in the Animals in Science and Society Unit of the Population Health Sciences Department at the Faculty of Veterinary Medicine, Utrecht University. It works closely with its the Animal Welfare Body, and has one full-time and four part-time advisors, who are employed on other projects. In contrast, *NCad* is supported by a secretariat consisting of a general secretary, a specific secretary for NCad and a senior policy advisor. NCad is financed by the Ministry of Agriculture, Nature and Food Quality and the secretariat is incorporated into the Netherlands Enterprise Agency, which is directly related to this Ministry.

Norway

In Norway, *Norecopa* has currently one full-time employee in its secretariat. An assistant works on regular updates and maintenance of two of Norecopa's databases (NORINA and TextBase), paid on an hourly basis. The Annual General Meeting approves the Board, which in turn delegates the daily work of Norecopa to the Secretary. Members of the Board contribute to many of Norecopa's projects and policy decisions, without receiving remuneration.

Poland

The Polish *CMA* is a part of the Department of Translational Research headed by Prof. Edyta Reszka. This Department was created in 2021 by the merging of the Department of Toxicology and Carcinogenesis and the Department of Molecular Genetics and Epigenetics. Since 2021, the head of CMA has been Dr Joanna Roszak, who continues the work of Dr Maciej Stępnik, together with three other researchers. The CMA was originally founded and financed as part of a governmental project. However, there has been no dedicated funding for the centre for the past few years. The co-workers are employed by the Department of Translational Research/NIOM, and any Three Rs activity in the CMA is undertaken on a voluntary basis or as part of other routine studies or projects. Money for reagents and equipment is saved by astute outsourcing, so that when financial support is obtained through research grants, statutory grants and commercial orders, any funds remaining after covering the existing financial requirements of the Department of Translational Research can be used by the CMA.

Portugal

The Three Rs centre at *i3s* in Portugal is currently being established and will include existing personnel from the institute's different research groups and scientific platforms. Most staff are employed on permanent contracts as either researchers or technicians within various parts of the institute, and they will undertake their role in the Three Rs centre as part of their existing job.

Romania

The Romanian Center for Alternative Methods (ROCAM) is focused on the dissemination of the Three Rs. In addition to conferences and workshops, partially presented in the previous article, ROCAM is providing Education and Training for both undergraduate and doctoral students. 'Biology breeding and pathology of laboratory animals' is a programme dedicated to veterinary students that includes ethics and a comprehensive Three Rs element, and 'Humane endpoints and harm–benefit analysis in experimental protocols on laboratory animals' is dedicated for the doctoral students. Nowadays ROCAM is in an advanced stage of implementation of a modular training programme on the

Three Rs and Laboratory Animals Science consistent with *Directive 63/2010/EU* Functions A, B, C and D, dedicated to PhD students in the area of biomedical science.

Slovak Republic

The Three Rs platform *SNP3Rs*, in the Slovak Republic, is led by four volunteers. The platform has about 25 active members in total, with a broad expertise relevant to the Three Rs. The Three Rs platform receives mostly non-monetary support from its members. The Ministry of Agriculture and Rural Development financially supported the development of the platform's website (www.snp3Rs.com), which became the main communication tool with the public. In Slovakia, there is no dedicated funding scheme available for the development and validation of new alternative methods or for the dissemination of information. However, the national funding programmes VEGA and APVV supported projects in 2019 and 2020 at the IEFT CEM and SAS, leading to the validation of alternative methods in the area of medical devices. Therefore, besides the activities leading to better and faster implementation of the Three Rs in education and science, one of the long-term goals of the platform is to secure funding from national sources. Such funds are necessary to support the activities and work of experts working on the implementation and fulfilment of requirements laid down in *Directive 2010/63/EU*.

Spain

At *CMCiB* in Spain (part of the Institut Germans Trias i Pujol (IGTP)), the in-house staff includes around 30 experts in the areas of animal welfare and husbandry, study design, biocontainment, surgery, quality and image diagnostics, and bioimaging, as well as in computational analysis methods and mathematical modelling. Collaborators include staff members from the IGTP itself, but also staff from the companies that collaborate with CMCib, such as Canon Medical Systems, ZeClinics and the UPC.

Sweden

The *Swedish 3Rs Center* is a part of the Swedish Board of Agriculture, but it has the National Committee for the Protection of Animals used for Scientific Purposes as its steering group. The National Committee also appoints an expert group to support the 3Rs Center's work. In total, nine employees work at the centre: an operations manager, one administrator, one communications officer and six administrative officers.

Switzerland

The *3RCC* in Switzerland has a complex organisational structure. It consists of: a Strategic Board, an Executive Board, a Management Team and a Directorate. The 3RCC also has an external advisory body in the form of the Scientific Advisory Board, composed of international Three Rs experts. The Strategic Board includes representatives of the 3RCC member institutions, as well as representatives of nominated observer members, which currently are SERI and the FOPH. It defines the strategic orientation and implementation of the centre, and decides about funding allocation to research projects based on recommendations from the Scientific Advisory Board. The Executive Board is composed of the 3RCC Executive Director, representatives of higher education institution members (referred to as Node Coordinators), and representatives of the Swiss Animal Facilities Network (SAFN), the Animal Welfare Officers Network (AWON), the Institute of Laboratory Animal Sciences (Institut fur Labortierkunde, LTK) and the Réseau des animaleries lémaniques (RESAL). The Executive Board is responsible for executing and implementing the 3RCC's strategy in the areas of research, education and

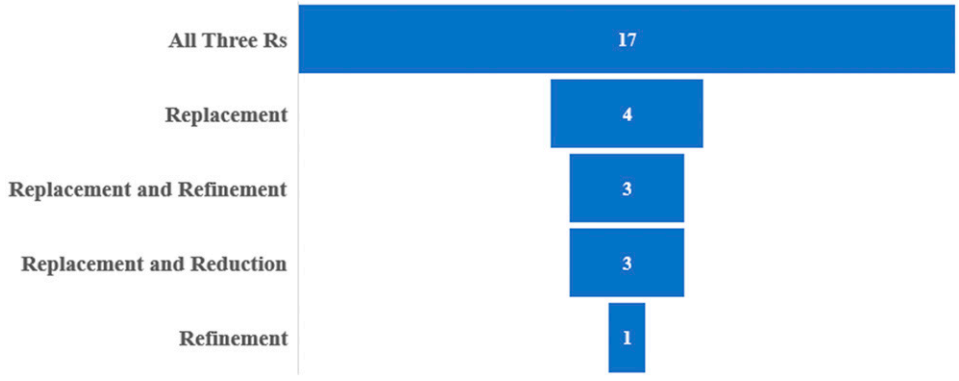

Figure 2. The core Three Rs areas covered by the individual centres and platforms. The graph shows main Three Rs focus of the featured Three Rs centres and platforms (total $n = 28$, no specific information on core Three Rs areas provided from NCad) in terms of the individual Three Rs, as well as their various combinations.

communication, with input from the Scientific Advisory Board. In addition, the Executive Board monitors progress in the implementation of the Three Rs principles in Switzerland and may contribute to specific Three Rs projects. The Scientific Advisory Board provides scientific expertise to the Executive Board and Strategic Board, assesses the progress and functioning of the centre, and evaluates the project proposals for the 3RCC funding programme. The Management Team fills the intermediary decisive position, supporting the Strategic Board on operative and budgetary decisions. The 3RCC's Directorate is the central coordinating organ that implements activities following the strategic lines as defined by the Strategic Board. The Directorate is composed of five staff: an executive director, two scientific officers, a communications officer and an administration and financial officer. All employees of the 3RCC Directorate are on permanent contracts at approximately 80% FTE.

United Kingdom

The *NC3Rs* in the UK is led by the Chief Executive who is advised by the NC3Rs Board (for further information see https://www.nc3rs.org.uk/about-us). The current staff number is 33, employed on indefinite contracts. NC3Rs activities are supported by a large number of academic and industrial researchers who give their time freely as members of grant panels, working groups and committees.

Ukraine

Finally, the *Ukrainian 3Rs Center* is led by a head and currently seven actively involved members, who work voluntarily and thus are not paid for their Three Rs activities.

Conclusions on the various organisational structures

In summary, there are very different organisational structures associated with the various Three Rs centres and platforms. The concepts presented here can, on the one hand, serve as examples of how a Three Rs centre can be set up, but also highlight the need for further financial support for some of the centres and platforms, in order to ensure their operational sustainability.

The core Three Rs areas covered by the individual centres and platforms

The Three Rs stand for the Replacement, Reduction and Refinement of animal use in experiments. Although the aim of most of the Three Rs centres and platforms is to cover all three areas, there are a few that focus specifically on one or two of the Rs. Figure 2 shows that 27 of the 28 centres and platforms focus on *replacement*, while 17 of them also work on *reduction* and *refinement*. Three cover *refinement* or *reduction* in addition to *replacement*, and four of the centres and platforms focus solely on *replacement*, clearly indicating that *replacement* is the area almost universally covered by the centres and platforms.

For example, the core area of TARCforce3R is *refinement*, since it is connected to a large animal facility. Specifically, improving housing conditions and setting up training programmes for the use of laboratory rodents is a major focus of their work. As TARCforce3R is also involved in the education and training of scientists, instruction in animal-friendly handling techniques and animal training methods is also a key area. Nonetheless, the other two Rs are also covered, but do not represent core areas.

With regard to specified core areas, the work at R2N focuses on the *reduction* and *replacement* of animal

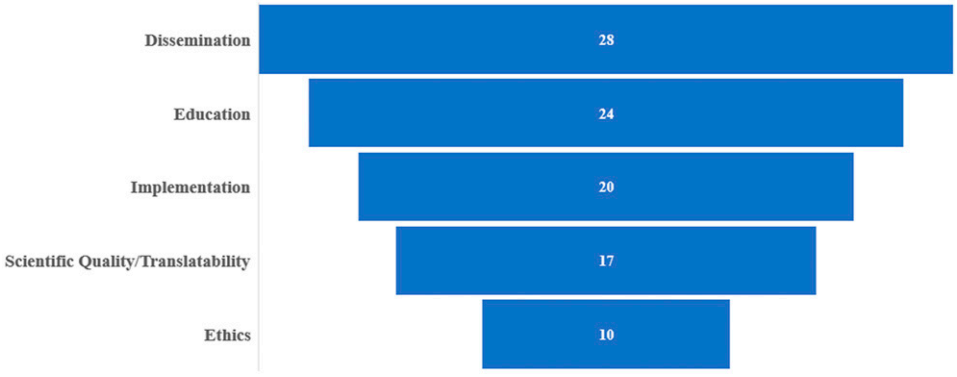

Figure 3. Three Rs-related topics that are that are covered by the centres and platforms. The graph shows the number of featured Three Rs centres and platforms (total n = 28) that cover the listed Three Rs-related topics as part of their ongoing activities (multiple options are possible for each centre/platform, no specific information from NCad included). These five topics were identified as having the most urgent need for action.

Table 2. Overview of some activities of the Three Rs centres and platforms relevant to possible collaboration opportunities.

Name of the institution	Country	Uses (and is able to provide) educational material on the Three Rs	Holds workshops or training on Three Rs methods	Has a searchable NAMs database	Organises public and regular Three Rs meetings	Has an *in vitro* laboratory available for collaboration	Is a member of EU-NETVAL	Funds research
RepRefRed Society	Austria	Yes	Yes		Yes			
Innovation Centre–3Rs (IC-3Rs)	Belgium	Yes		Yes	Yes	Yes		
3Rs Centre Czech Republic	Czech Republic	Yes	Yes			Yes	Yes	
The Danish 3R-Center	Denmark	Yes			Yes			Yes
BB3R–Freie Universität Berlin	Germany	Yes	Yes		Yes			
CAAT Europe	Germany	Yes	Yes	Yes	Yes	Yes		
Charité 3R	Germany	Yes	Yes		Yes	Yes		Yes
Einstein Center 3R	Germany	Yes	Yes		Yes	Yes		Yes
ICAR3R–3R Centre JLU Giessen	Germany	Yes	Yes		Yes			
Leibniz Alternatives at IUF–Leibniz Research Institute for Environmental Medicine	Germany	Yes				Yes		
R2N	Germany	Yes			Yes	Yes		
TARCforce3R	Germany	Yes	Yes		Yes			
3R Center Rhine Neckar	Germany		Yes		Yes			
VZET Hannover	Germany	Yes	Yes		Yes	Yes		
Trinity College Dublin	Ireland	Yes	Yes			Yes		
Centro 3R	Italy	Yes	Yes		Yes	Yes		
Luxembourg 3Rs Platform	Luxembourg					Yes		
NCad	Netherlands	Yes						
3Rs Centre of the Utrecht University and University Medical Centre Utrecht	Netherlands	Yes	Yes	Yes	Yes	Yes		

(continued)

Table 2. *(continued)*

Name of the institution	Country	Uses (and is able to provide) educational material on the Three Rs	Holds workshops or training on Three Rs methods	Has a searchable NAMs database	Organises public and regular Three Rs meetings	Has an *in vitro* laboratory available for collaboration	Is a member of EU-NETVAL	Funds research
Norecopa	Norway	Yes	Yes	Yes	Yes			Yes
National Center for Alternative Methods to Toxicity Assessment (CMA)	Poland	Yes	Yes			Yes	Yes	
i3S	Portugal	Yes	Yes		Yes	Yes		
ROCAM	Romania	Yes						
Slovak National Platform for 3Rs–SNP3Rs	Slovak Republic	Yes	Yes					
CMCiB-IGTP–Comparative Medicine and Bioimage Centre of Catalonia, Germans Trias i Pujol Research Institute	Spain	Yes	Yes			Yes		
Swedish 3Rs Center	Sweden	Yes	Yes		Yes			
3RCC	Switzerland	Yes	Yes		Yes			Yes
National Center for the 3Rs (NC3Rs)	UK	Yes	Yes					Yes
The Ukrainian 3Rs Center	Ukraine	Yes				Yes		

experiments in biomedical research, as does the 3Rs-Centre in Utrecht. In the case of the 3Rs Centre Czech Republic, most of the work focuses on *replacement*, but thanks to close co-operation with the Czech Laboratory Animals Association (CLASA), as well as involvement in the activities of the Central Commission for Animal Protection of the Czech Republic and the National Committee for the Protection of Animals at the Ministry of Agriculture, *reduction* and *refinement* are also addressed on a regular basis.

As an example of a Three Rs institute that covers all Three Rs, the RepRefRed Society in Austria tries to attach equal importance to each of the Rs, instead of focusing only on one R. RepRefRed Society officially wants to avoid taking either a position of endorsing animal experiments or of promoting alternative methods — they want to combine both positions and get the best out of it. The UK's NC3Rs also supports all Three Rs across all of its programmes, including research and innovation funding, early career awards and those activities led by NC3Rs staff.

Similarly, Norecopa works to support all Three Rs. For example, the NORINA database[11] — which consists of approximately 3000 alternatives to animal use in education and training at all levels from school dissections, through undergraduate education, to the training of laboratory animal staff and scientists — is an important contribution to *replacement*. Norecopa provides web-based information on experimental design and planning of animal experiments,[12] which contributes to all Three Rs. This information includes the PREPARE guidelines[13] for planning animal research and testing (https://norecopa.no/PREPARE). The recent creation of a Refinement Wiki[14] covers the area of *refinement*. In addition, Norecopa awards a 3R Prize[15] to recognise advances within all the Three Rs. This prize has been awarded every year since 2010, except for 2018 and 2020.

Overview of Three Rs-related topics covered by the centres and platforms

Clearly, the organisers of the various European Three Rs centres and platforms have identified the following topics as having the most urgent need for action within the Three Rs sector in general: dissemination of information; implementation; education; scientific quality/translatability; and ethics. Figure 3 summarises the extent to which these major topics are covered by the Three Rs centres and platforms. All 28 Three Rs centres and platforms are actively working on dissemination of Three Rs information. Of these, 24 are involved in educational activities, 20 contribute to the implementation of the Three Rs, and 17 participate in programmes about scientific quality/translatability. Ethics within the Three Rs field are addressed by ten Three Rs centres and platforms, as a separately identified working area.

Detailed activities of the Three Rs centres and platforms

This section describes in detail the individual activities of the European Three Rs centres and platforms within the identified priority topics listed above. Table 2 gives an overview of some of the activities being undertaken, which might be relevant for potential collaborative partners.

Austria

The Austrian *RepRefRed Society* contributes to dissemination of Three Rs information through its website, LinkedIn page and Facebook page, as well as its Three Rs online seminar series, which is hosted and organised by the two assistants of the board. The society also contributes to dissemination of Three Rs information by its participation in meetings and plenary discussions, and through its membership of other Three Rs organisations and networks. In addition, the RepRefRed Society distributes a newsletter to inform about events. To integrate the Three Rs into education, the RepRefRed Society organises events, like the '3R Days' for researchers and animal care personnel, and co-operates with a national organisation that trains teachers to undertake the role of Animal Protection Officer within nurseries, primary schools and high schools. The RepRefRed Society also informs about animal experiments, alternative methods to animal testing, legislation and the Three Rs.

In terms of implementation, the RepRefRed Society works on the harmonisation of continuing education and training on Three Rs methods, e.g. Laboratory Animal Science EU Function courses on carrying out procedures on animals according to §19 TVG 2012 (the Austrian *Animal Experimentation Act 2012*) and EU *Directive 2010/63* (with a focus on the national harmonisation of teaching contents).

In the field of scientific quality/translatability, RepRefRed runs projects at the Division of Biomedical Research at the Medical University of Graz, to promote among researchers the concept of sharing 'surplus' laboratory animals from a study, as an alternative to euthanising them.

Belgium

IC-3Rs (Belgium)[16] contributes to the dissemination of Three Rs information via its annual IC-3Rs Symposium, which focuses on interesting Three Rs-based research projects, and the yearly organisation (together with the IVTD)[17] of two international courses at VUB[18] on dermato-cosmetic sciences in the EU[19,20] with a focus on animal-free

methodology and legislation (which they have been organising now for over 25 years).

Regarding information dissemination, communication and training, IC-3Rs partners in EU projects (ONTOX, Twinalt), organises sessions at congresses such as the WC11 ('NASH, the liver disease of the 21st century? Alternative technology in the spotlights', chaired by V. Rogiers (BE) and S. Malany (USA)), and participates in congresses, workshops, symposia, etc., as co-organiser, speaker or with posters. The IC-3Rs also acts as Co-Chair of the Scientific Committee on Consumer Safety (SCCS), member of the EURL ECVAM Network for Preliminary Assessment of Regulatory Relevance (PARERE), European Partnership for Alternative Approaches to Animal Testing (EPAA) and the SCCS. In addition, it maintains RE-Place as a central database for *replacement* methodology based on the expertise of Belgian researchers and institutes; universities and companies are regularly visited to stimulate scientists to register their *in silico* and *in vitro* research on this database. During the COVID-19 pandemic, educational videos were made available in English, Dutch and French.

In the field of scientific quality/translatability, the focus of the IC-3Rs is on the development of animal-free methodology at the IVTD laboratory. Here, human stem cells are differentiated to hepatic cells, in order to study drug-induced liver injury. In addition, research is ongoing with respect to Next Generation Risk Assessment for genotoxicity testing and the development of alternative methods that can be used for the safety testing of medical devices. Moreover, the new methodology GENOMARK has been developed to de-risk false positive genotoxicity data based on a set of specific biomarkers. The methodology can be used with either qPCR or transcriptomics data, and the method was recently presented to EURL ECVAM (EU Reference Laboratory for alternatives to animal testing) for validation in 2022.

Czech Republic

The *3Rs-Centre Czech Republic* is involved in several dissemination activities. The dissemination of the Three Rs concept is realised by active participation at national and international conferences and workshops — for example, those organised by ESTIV, EUSAAT, SETOX (Slovak Toxicology Society), PROKOS (Association of Producers, Importers and Distributors of Cosmetics and their Ingredients) and CLASA (Czech Laboratory Animal Science Association), as well as the World Congresses on Alternatives and Animal Use in the Life Sciences. Members of the 3Rs-Centre are involved in international societies, such as EUSAAT (Dr Jírová as former vice-president) and ESTIV (Dr Dvořáková as board member), and serve as experts for the OECD, ECHA and EURL ECVAM.

With regard to the education aspect of the Three Rs, members of the 3Rs-Centre frequently give university lectures on alternatives (for example, at Charles University in Prague, Tomas Bata University in Zlin, and at the Czech Technical University and the University of Chemistry and Technology in Prague), and participate in the 'Training course for acquiring qualification and professional competence in the field of experimental animal use', with lectures on the Three Rs and ethics of animal experimentation. In the NIPH laboratories, at the Centre of Toxicology and Health Safety, the implementation of *in vitro* toxicological methods as alternatives to animal testing, and their optimisation for certain purposes — for example, medical devices and cosmetics — has currently been realised in compliance with ISO standards, accreditation and GLP quality systems. These *in vitro* toxicological methods are used on a regular basis to identify potential hazard or to confirm the safety of chemical preparations, cosmetics, medical devices, or other consumer products. The 3Rs-Centre serves as an advisory body for interested parties, and can offer demonstrations or short training sessions, to facilitate the successful transfer of *in vitro* methods to other laboratories. Members of the 3Rs-Centre participate in the authorisation of experimental projects, in co-operation with the Animal Welfare Committee at the Ministry of Health, ensuring that the approved projects fulfil the Three Rs principles required by *Directive 2010/63/EU*.

With regard to the topic of scientific quality/translatability, in 2013, the Ministry of Agriculture of the Czech Republic nominated the National Institute of Public Health in Prague as the contact point to provide advice on the regulatory relevance and suitability of alternative approaches proposed for validation (PARERE); the National Reference Laboratory for Experimental Immunotoxicology, at the Centre of Toxicology and Health Safety, was nominated as a specialised national reference laboratory to join the network of selected national reference laboratories (EU NETVAL). Following the selection procedure, the National Reference Laboratory was successfully appointed to join the network. EU NETVAL laboratories participate in the validation of alternative *in vitro* methods under the supervision of EURL ECVAM. Currently, the National Reference Laboratory is collaborating in a project focused on the validation of thyroid hormone disruption methods and developing the method 1b–TSH receptor-mediated activation.

The topic of ethics is covered by lectures on the ethics of animal experimentation, which are presented in the scope of the 'Training course for acquiring qualification and professional competence in the field of experimental animal use'.

Denmark

Dissemination is the main focus in the daily work of the *Danish 3R-Center*, for example, via the websites www.3rcenter.dk, www.natud.dk (website for the Danish national committee), and the organisation of an International

3R-symposium. Another important part of the Danish 3R-Center's work is to provide financial support to Three Rs research projects, on behalf of the Ministry of Food. Once a year, the Danish 3R-Center calls for applications for research projects that, in one way or another, have the potential to improve the laboratory animal area through *replacement*, *reduction* or *refinement*.

Germany

ICAR3R: The scientific focus of ICAR3R is on *refinement* and *replacement*, in order to develop joint strategies in an interdisciplinary approach that are able to reduce the number of laboratory animals used. The aim of research into *refinement* is to develop procedures that can avoid potential harms during animal experiments in the most effective way. *Refinement* research thus makes an active contribution to applied animal welfare. In order to be able to interpret clinical and behavioural signs of stress in animals under experimental conditions, the centre prioritises research into the development of valid stress assessment criteria, in order to help identify effective *refinement* measures or criteria for termination. This also includes the intensive training of all persons involved in animal experiments (e.g. established researchers, young scientists, students, animal care technicians, technical staff) so that they are able to recognise signs of potential stress at an early stage. At the same time, the development of *replacement* methods is being advanced — for example, by developing complex and biologically realistic computer models of cells in healthy and diseased tissue (*replacement* research). The computer models developed are used to test scientific hypotheses *in silico* before they are tested *in vitro* (e.g. in a cell culture) or *in vivo*. To reduce the numbers of animals used in experiments, computer simulations are combined with tissue-based *in vitro* systems (e.g. organotypic slice cultures). The focus is particularly on data-based computer models of nerve cells and their networks. The fast and effective transfer of knowledge and new simulation methods and models to the scientific community is ensured by publication output and the online accessibility of the computer models that have been created (e.g. *ModelDB*: https://senselab.med.yale.edu/modeldb; *Neuromorpho*: http://neuromorpho.org).

Another important pillar of ICAR3R is Three Rs education. The infrastructure of its associated universities offers excellent opportunities to develop new didactic strategies, in close co-operation with various departments, to implement the Three Rs concept in a sustainable way. The results of the centre's work, and the close networks with other national and European Three Rs centres, form the prerequisite for the necessary scientific exchange and for the effective communication of Three Rs information. As examples of initiatives in the fields of education, dissemination and ethics, the following can be listed:

- the ICAR3R website (https://www.uni-giessen.de/fbz/zentren/icar3r), which is financed by the Justus-Liebig-University Giessen and is part of the website of the Justus-Liebig-University Giessen;
- the LIVE3R website (https://www.uni-giessen.de/fbz/zentren/icar3r/projekte/live3r), which is financed by Kogge-Foundation and is part of the website of the Justus-Liebig-University Giessen;
- a series of lectures on Animal Welfare and Ethology I, II and III (for students of Veterinary Medicine);
- a course on Laboratory Animal Science (for students of Veterinary Medicine);
- several elective courses, namely, 'Discussion platform on Animal Welfare', 'Species and nature conservation seminar', 'Animal welfare with a difference: A scientific look at current animal welfare issues' and 'Occupational field exploration animal protection: Official veterinary activity';
- interdisciplinary training on 'Aspects of animal welfare in science' within the Agricultural Master's Programme, and 'Legal module of the ecological toolbox' within the Biological Master's Programme on Ecology, Evolution and Conservation;
- the lecture series 'Just.Us and Animal Welfare' for veterinarians and interested scientists;
- laboratory animal science courses focusing on *refinement* and Culture of Care, in which participants gain expertise to perform experimental procedures on animals;
- a 3R Symposium held every two years (the first one was in 2018, followed by a second one in 2020), including the award ceremony of the Ursula M. Händel Animal Welfare Prize of the DFG (German Research Foundation);
- a 3R Skills Lab;
- a Certificated Course on 'Animal assisted services';
- an ethics workshop, entitled 'The Three Rs principles and the balancing of interests in the context of ethics, law and practice' (self-financing);
- an advanced training farm for use as a classroom by teachers at all school levels, people working in education, students and the interested public;
- a workshop on 'Medical training in theory and practice' for trainee animal care technicians, offered by ICAR3R and the Hessian Ministry for the Environment, Climate Protection, Agriculture and Consumer Protection, under the patronage of the State Animal Protection Commissioner of Hesse (Dr Madeleine Martin);

— a workshop on 'Medical training for zoo animal keepers', offered by ICAR3R and the Hessian Ministry for the Environment, Climate Protection, Agriculture and Consumer Protection, under the patronage of the State Animal Protection Commissioner of Hesse;
— a workshop on 'Medical training for the examinants' (self-financing).

With regard to the implementation of the Three Rs, ICAR3R has a 3R Skills Lab to provide training for personnel who work with laboratory animals and to help firmly establish Culture of Care principles. ICAR3R are also conducting a study on the implementation of *refinement* methods in the use of a number of animal models, and building up the above-mentioned LIVE3R website. Another essential pillar of ICAR3R is the complex and interdisciplinary analysis of 'Culture of Care', at all levels in animal research.

CAAT-Europe: This centre works hand-in-hand with its sister centre, CAAT at Johns Hopkins University, Baltimore (MD, USA), created in 1981.[21] These centres aim to provide a platform for communication and networking among stakeholders from industry, regulatory agencies, NGOs and academia, focused on developing new concepts in toxicology. Examples of communication channels are workshops, information days, symposia and scientific sessions at international conferences. To date, CAAT-Europe has organised around 50 high-level workshops and more, including the activities organised in the context of individual projects. A powerful collaboration tool applied by CAAT is the publication of scientific papers. To date, the centre has authored and coordinated the publication of more than 100 scientific works, involving ~700 co-authors from different stakeholder groups.[22,23] These activities have contributed to creating a broad critical mass, crucial for developing strategic projects and promoting human science and NAMs in toxicology and biomedicine. CAAT and CAAT-Europe's official communication organ is the open-access *ALTEX* journal. Via this channel, the centres contribute to the following series:

— *Food for Thought*: articles allowing authors to describe their ideas of where a section of the field of alternatives to animal experiments should be heading.[24–27]
— *Bench Marks*: a series focused on hands-on issues in the everyday laboratory *in vitro* work. The articles are concise and practical. This series has been introduced to standardise common practices and is aimed at researchers, but also industry, regulators and policymakers, due to its compact and straightforward format.[28–31]
— *Think tank reports and workshop reports*: detailed reports or road-maps, as a product of the discussion that occurs at a think tank meeting or workshop.[32–35]
— *Corners*: overview of highlights, news and events organised by CAAT and its projects (https://www.altex.org/index.php/altex/article/view/2464/2392).

The centres jointly disseminate through the CAAT website (http://caat.jhsph.edu) with more than 18,000 visitors per month. The electronic CAATwalk newsletter (https://caat.jhsph.edu/publications/Newsletter/) has more than 8000 recipients, CAAT's Facebook account has more than 12,000 followers.

Concerning the education programme, a joint programme is run between Johns Hopkins and the University of Konstanz, with student exchanges, e-learning and collaboration to international graduate school (KORSB–chemical biology). Besides various university classes, CAAT offers a range of courses on Coursera free of charge, such as: 'Toxicology 21st century'; 'Scientific applications' (over 8000 active learners by May 2022); and 'Evidence-based toxicology' (over 6000 active learners). Being science-driven research centres, CAAT and CAAT-Europe have been involved in joint applications and strategies for collaborative national and European research projects, setting up transatlantic consortia for international research projects on alternative methods. Some most recent examples are the toxicological research initiatives EU-ToxRisk, RISK-HUNT3R, ONTOX and PrecisionTox funded by the EC H2020 programme, and working together under the ASPIS cluster umbrella (https://aspis-cluster.eu/). A recent initiative has started focusing on the promising technology of micro-physiological systems (MPS). The MPS and systems toxicology programme have started a series of MPS world summits (https://mpsworldsummit.com), which, after New Orleans in 2022, will come to Berlin in 2023. CAAT has a leading role in the development of concepts and guidelines, such as: Good Cell Culture Practice (GCCP; just published version 2.0);[36] Good Read-Across Practice (GRAP);[37] Green Toxicology;[38] Evidence-based Toxicology Collaboration (http://www.ebtox.org); and the annotated toxicity test method template, ToxTemp.[39] CAAT also runs a *refinement* programme.

In the field of scientific quality/translatability, CAAT and CAAT-Europe work on developing alternative methods through the associated laboratories in Konstanz and Baltimore, focusing on developmental neurotoxicity. Furthermore, CAAT entertains a policy programme through ALTERTOX at the European Parliament. CAAT-Europe is an official external expertise service provider in the area of science and technology options assessment for the European Parliament, and offers a lobbying platform for the best available science.

3R-Center Rhine-Neckar: The activities of this centre are structured within four animal welfare pillars. The first pillar concerns its role as a coordination centre for Three Rs

projects within the Rhine-Neckar region — maintaining a central animal experiment database, as well as organising education and support for researchers between all partner sites. In more detail, 3R-Center Rhine-Neckar is enhancing the coordination of animal experiments in the Rhine-Neckar region via a central animal experiment database, to help avoid duplication of experiments and to coordinate terminal experiments with organ harvesting. For this purpose, it is collaborating with AniMatch (animatch.eu), which is an online sharing platform that facilitates the exchange of organs and tissues of laboratory animals used for scientific purposes. AniMatch provides a simple, efficient way for researchers to make a change toward less animal use. Due to the dense proximity of basic and preclinical research sites in the region, there can be a very efficient sharing of resources. On the education and support side for researchers, the centre provides in-depth training in the Three Rs principles, providing scientists with specific and available resources to avoid animal research, and also the knowledge to help choose the models with the greatest promise of addressing particular research questions.

In the second pillar, the centre aims to increase the accessibility and transparency of animal research in the Rhine-Neckar region through Open Science, and it also promotes research data management. Free access to the results of scientific work is one of the central aims of Open Science. It comprises publishing research in Open Access journals as so-called 'Gold' Open Access, as well as contributing to Open Data, i.e. the publication of the original research data, so that they can be accessible for replication and re-use by others. Additionally, the DFG proposes guidelines on handling research data, that outline three important issues to be taken into account, namely that: research data management needs to be planned; access to data should be granted to others; and long-term archiving of the data is required. These are not only issues of good scientific practice, but also support the reproducibility of research and FAIRness of research data (making data Findable, Accessible, Interoperable and Reusable).[40] In order to make research data management and Open Science an integral part of the 3R-Center Rhine-Neckar, an education platform is provided on the website (www.3r-rn.de) to explain how to find the right journal (Directory of Open Access Journals), how to get the publication costs reimbursed, how to convert animal data according to the FAIR guiding principles and, finally, where to store scientific data appropriately.

The third pillar is represented by the centre's role as the single point of contact for the design of new experiments in the Rhine-Neckar region, with the main aim of enhancing basic and preclinical research quality and conveying quality standards in biomedical research. The centre only considers research on animals to be ethically justifiable, if it generates value for science and society. The position paper by Strech and Dirnagl[41] proposes three additional measures (robustness, registration and reporting) to increase the scientific validity of the results and maximise the knowledge gained from each experiment. To help implement these three measures, the centre assists researchers in the planning and registering of studies — with study pre-registrations being carried out via the centre's partner site within the German Federal Institute for Risk Assessment. It also supports researchers in the generation of robust data, through the implementation of the PREPARE guidelines (Planning Research and Experimental Procedures on Animals[42]) and by providing reporting guidelines such as ARRIVE (Animal Research: Reporting of *In Vivo* Experiments[43]) for basic and preclinical data. These and other resources are collated within the '3R-Rhine-Neckar Toolbox' on the centre's website, to simplify the retrieval of relevant, high-quality information and to facilitate the implementation of these guidelines.

The fourth pillar provides targeted research activities at the CIMH and the Mannheim Faculty of Medicine, to develop measures aimed at improving the welfare and treatment of animals used in research, as well as the development of alternatives to animal research. In order to refine animal welfare and treatment, the centre is currently conducting two research projects. The first project focuses on the implementation of a social behaviour system for rats (ActualHCA System), which enables tracking of individual behavioural data (locomotion, social interaction, eating, drinking) in a group-housing condition. The implementation of this system will make experimental settings based on the single housing of rats (e.g. when measuring behavioural endpoints) redundant and will therefore be a milestone in improving animal welfare. The second project focuses on the development of a non-invasive central nervous system drug delivery system. Since the blood–brain barrier represents a major hurdle for target engagement of many new treatment drugs in preclinical research that either need very high peripheral invasive dosing (e.g. intraperitoneal) or surgical applications (intracerebroventricular), the centre aims to develop an intranasal application system, that can bypass the blood–brain barrier, and efficiently and precisely deliver therapeutic molecules to the central nervous system. In addition to these two experimentally driven research projects, the centre is implementing *in silico* approaches to replace animal experiments. For example, in the past five years, the centre has developed the open access databases Syphad (http://www.syphad.org) and ChemNetDB (http://chemnetdb.org), consisting of literature-based data sets from more than 150,000 rats. With these data sets, the centre has built a neurochemical connectome of the rat brain, as well as neurotransmitter response patterns of neuropsychiatric drugs.[44,45] By applying chemoinformatics to these big data sets, the centre is able to make predictions on brain-wide neurochemical and molecular response patterns of new drugs, and only a few validation experiments are then needed to verify the *in silico* predictions.

R2N: The R2N website (https://r2n.eu) is used as a main vehicle for dissemination of its activities. R2N was also part of the Ideas Expo 2019, where students and teachers were informed about R2N's work. Members of R2N publish their research data in peer-review journals. With regard to education, R2N organises internal symposia every year, where the progress of all research projects being carried out within R2N is presented. There are also 2R winter camps held, to support the networking between project groups.

Leibniz Alternatives: This platform hosts its own website (https://en.leibniz-alternatives.de), where its projects and publications are displayed. Moreover, it intends to disseminate data through DNT-DIVER (hosted by the US National Toxicology Program; NTP) and the US Environmental Protection Agency (US EPA)-hosted ToxCast databases. With regard to education activities, Ellen Fritsche, head of Leibniz Alternatives, teaches about alternatives to animal testing, developmental neurotoxicity (DNT) and the AOP concept at the Heinrich-Heine University Dusseldorf and the RWTH Aachen. Within the DNT field, Ellen Fritsche is actively involved in the implementation of the DNT-IVB (developmental neurotoxicity *in vitro* battery) into regulation. Here, she collaborates with EFSA, the Danish and US EPAs, the US-NTP and the OECD. Leibniz Alternatives is very active in the core area of scientific quality/translatability. The Ellen Fritsche laboratory developed the 'Neurosphere Assay' as part of the above-mentioned *in vitro* battery for DNT testing. Within an EFSA-funded project, the laboratory is part of an international consortium that has assembled, established and scientifically validated a test battery for predicting the effects of substances on the developing nervous system more quickly and cost effectively without the need for animal testing.[46] These data form the basis of a currently prepared OECD guidance on use and interpretation of the DNT-IVB.

Currently, methods for evaluating embryotoxicity (hiPS test),[47] which is based on the ability of human induced pluripotent stem cells (hiPSCs) to differentiate into beating cardiomyocytes and test methods for evaluating adult neurotoxicity by using neurally-induced hiPSC, are being developed within the CERST-NRW project funded by the Ministry for Culture and Science of the State of North-Rhine Westphalia, Germany. CERST-NRW also funds the set-up of a biostatistical evaluation pipeline for the transparent analysis of screening data in collaboration with the Brunel University, London. Furthermore, with CERST-NRW funding, quality control standards for work dealing with hiPSCs in an academic setting were proposed and applied.[48] More glia-related test methods are on their way within the Cefic-LRI project, filling gaps in the current DNT-IVB. Within the ENDpoiNTs (H2020) project, there are test methods set up to assess endocrine disruption (ED)-related DNT, that also complement the current DNT-IVB.[49] The DNT-IVB is also included in the ONTOX (H2020) project aiming to implement Three Rs methods by creating NAMs for human risk assessment of chemicals without the use of animals, in line with the principles of 21st century toxicity testing and next generation risk assessment.[50] ONTOX builds upon the probabilistic risk assessment approach that improves the regulatory confidence in NAMs by assessing the uncertainties introduced by each step, including the probability of hazard and exposure. Mechanistic knowledge of NAMs — based on human physiology and described by quantitative Adverse Outcome Pathways (AOPs) — and an assessment of their plausibility, are crucial for their regulatory acceptance and the necessary shift from animal-based methods.[51] Through the use of cell-based results, all projects carried out at the Leibniz Alternatives contribute to the development of AOPs for regulatory application.[52–58]

TARCforce3R: The website (https://www.unimedizin-mainz.de/tarc-force-3r) gives information on their projects, their courses on animal handling and training, and information about the rehoming of animals. TARCforce3R is involved in the laboratory animal courses at the University Medical Centre Mainz, which are also implemented as elective courses in Bachelor and Master programmes. TARCforce3R also provides instruction on animal-friendly handling, and regularly trains animal facility staff. It also provides training and workshops within other institutions. Moreover, it regularly presents its work at animal welfare and laboratory animal-specific conferences. TARCforce3R advises scientists regarding the practicalities of the Three Rs and on the implementation of *refinement* techniques in their animal facilities. One of its key areas is to establish training strategies for small rodents, which aim to reduce their stress levels and thus help improve the quality of the science.

BB3R: The BB3R has its own website, featuring the project partners and their publications, Three Rs-related events (organised by BB3R and others) and information about their Three Rs activities. BB3R organises annual Spring Schools (with a focus on non-animal methods, *refinement* and ethics/law) and an annual seminar series, 'Alternatives to animal use in research and education — Refine, Reduce & Replace'. A detailed description of the projects from the BB3R members can be found on the BB3R website (www.bb3r.de).

Currently, the Three Rs focus of the FU Berlin Department of Pharmacology is on the integration of immune cells into human skin models. Additional research focuses

on models of tissue vascularisation. The results of detailed anatomical studies, as well as the quantification of stress hormones, will allow *refinement* in studies on mice and minipigs. Furthermore, the modelling of blood vessels *in vitro* paves the way for *replacement* opportunities in the future.

Within the framework of the Excellence Cluster 'Science of Intelligence', algorithms are being derived from the behaviour of mice that represent intelligent behaviour. Here, for example, a tool is being developed to automatically evaluate the facial expressions of mice (Mouse Grimace Scale) in images, by applying methods based on artificial intelligence. Along these lines, FU Berlin researchers aim to investigate 'Choice tests as a means for severity assessment from an animal's point of view' and 'Cognitive bias test as a means for severity assessment from an animal's point of view' in projects funded by the DFG research group on Severity Assessment.

Notably, FU Berlin is involved in the development of innovative products for humane teaching approaches, by combining user surveys and cutting-edge techniques — for example, in the Bf3R-funded project 'Simul-RATor', an interdisciplinary team of FU Berlin researchers are developing an anatomically correct and cost-effective simulator for laboratory animal science courses. From the results of user and teacher surveys, the team aims to optimally adjust the simulator to fit each course application. All results will flow into a final requirements analysis, on the basis of which a new simulator will be created at the Institute of Veterinary Anatomy by using 3-D printing.

Charité 3R: Their activities focus on communication, education and support, and research. Regarding communication, Charité 3R presents Three Rs research projects and many additional resources on its website (https://charite3r.charite.de/), it holds scientific symposia, events for the public, and provides flyers and video productions on Three Rs applications. For the education of PhD students, Charité 3R organises, twice a year, a one-week '3R curriculum' on Three Rs applications and ethics of biomedical research. This is accompanied by regular 'ReThink3R' workshops (rethink3r.de), which invite young investigators to develop practical solutions for the implementation of the Three Rs principles by using Design Think methods. In addition, the Charité 3R Toolbox[59] provides a comprehensive online information resource that is structured according to the Six Rs — including the three additional Rs of Robustness, Registration and Reporting.[60] To support Three Rs-related research, Charité 3R provides calls and internal funding for Three Rs research projects and infrastructure at Charité. Further activities include surveys on Three Rs implementation among Charité researchers, and support for third-party funding applications. Charité 3R also supported the application for the Einstein Center 3R (EC3R), which connects the relevant research institutions in Berlin, including FU Berlin, HU Berlin, TU Berlin, MDC, RKI, BfR and Charité.

EC3R: This recently-established centre has a clear scientific focus on the development of human-relevant NAMs. Six research projects are being undertaken, to develop organ models for the lung, liver, heart, brain, neuromuscular junctions and intestine. The research activities are supported by two cross-sectional projects. The Six R project, which adds Robustness, Registration and Reporting to the well-known existing Three Rs, aims to improve the reliability of 3-D organ models; the second project investigates the cross-species comparability and validity of disease models. Newly developed human organ models will be provided to user groups for research purposes, to disseminate the use of NAMs. In education, EC3R brings together the existing educational activities of the participants, expands them, and makes the resources more freely accessible. Webinars and research groups led by junior scientists/professors introduce Three Rs methods to young scientists. At the same time, the centre serves as a joint Berlin communication platform on the topic. The aim is to inform, in a scientifically sound and fact-based manner, on the current state of research.[8] This will help to disseminate knowledge on the newly developed technologies, while at the same time avoiding false expectations. More information about its activities is available on its website, https://www.ec3r.org/en/.

VZET: Information and resources are disseminated via its website (www.tiho-hannover.de/vzet; Table 1), where it presents its scientific activities and exchanges information about the *in vitro* methods being used to reduce the number of animal experiments at the TiHo. VZET is also part of the 3R Smart project — the 3R Smart website (https://www.3r-smart.de/) is an information and training platform on alternative and supplementary methods to animal experimentation, financed by BMBF (2018–2023).

Regarding education, VZET offers courses for students of Veterinary Medicine on laboratory animal science (FELASA B and C), including a lecture on the Three Rs and a workshop on searching strategies for alternatives to animal experiments in project planning. The courses are designed to maximally reduce any stress in the animals used, and this is achieved through the preliminary use of dummy models from the clinical skills laboratory (CSL) at the TiHo. Furthermore, VZET offers courses on the basics of alternatives to animal experiments in toxicology, the skin corrosion testing of chemicals *in vitro*, and basic cell culture, including insights on GIVIMP and good cell culture practice (GCCP), as well as a course on the potential implementation of the Three Rs in science.

The CSL offers the opportunity for students of Veterinary Medicine to learn an extensive range of practical skills through the use of dummy models — for example, intravenous injections, blood sampling, suturing techniques, surgical activities and rectal examinations. This training avoids subjecting animal patients to unnecessary stress during student exercises, due to the students not yet having adequate practical skills. It also results in a reduction in the numbers of animals used for teaching purposes.

With regard to scientific quality/translatability, Three Rs-related research in the VZET is widespread; a full list of Three Rs projects and publications can be found on its website (https://www.tiho-hannover.de/vzet). Many VZET members are involved in the R2N research network, mainly with infection studies and projects related to the One Health concept.

The teaching of animal ethics in aetiological and clinical subjects at the TiHo has been integrated into the concept of 'critical supervision' of, for example, PhD theses or doctoral and Master/Bachelor studies. Thus, the TiHo has entered new territory in several respects. It supports the implementation of ethical content within study programmes, wherever and whenever corresponding questions and ethical dilemmas arise for the students. With its activities, TiHo won the Global Animal Welfare Award in 2022.

Ireland

At *TCD* in Ireland, to disseminate information on the Three Rs they use their monthly newsletter, which is distributed to all scientists working with animals at the university. TCD has joined the International Culture of Care Network[61] and members of TCD participated in several Three Rs-relevant publications and occasions. For example, Adriele Prina-Mello and Dania Movia are Guest Editors for the *Animals* journal, under the topic 'Replacing animal testing in research — A scientific & regulatory perspective of current efforts towards the 1R'. They have also co-edited an e-book published by *Frontiers in Bioengineering and Biotechnology* entitled 'Use of 3D models in drug development and precision medicine'.

In July 2020, Dania Movia was among the 77 scientists that, in collaboration with Animal Free Research UK, Safer Medicines Trust and Alliance for Human Relevant Science, signed an open letter (published in *The Guardian*) calling for a paradigm shift and a clear timetable for regulatory change to enable an accelerated drug development without animal use (https://www.animalfreeresearchuk.org/openletter/). José M. Sánchez Morgado and Aurora Brønstad edited the first volume in the Laboratory Animal Science and Medicine series, entitled 'Experimental design and reproducibility in preclinical animal studies'. The results of *in vitro* alternatives to animal studies were presented at the ISAM 2021 Congress, at the University of Cambridge, at University College London, and at the ERS Research Seminar.

With respect to the activities of TCD to promote the Three Rs in education, TCD held the 2019 Trinity College Dublin Winter School on Systematic Reviews and the 2020 Trinity College Dublin Winter School on Experimental Design and Statistics. TCD organises a Three Rs course for all Trinity's scientists. It has also worked on a document about the development of guidance on core and Function A, and contributed to the ETPLAS working group report, which was published as Dontas IA et al., 'Assessable learning outcomes for the EU Education and Training Framework core and Function A specific modules: Report of an ETPLAS Working Group'.[62] The LBCAM at TCD has introduced educational aspects of the Three Rs and the application of new approach methodologies (NAMs) to preclinical studies within the Trinity College Master's degree in Molecular Medicine, under the Precision and Translational Nanomedicine module. TCD is currently building the first Irish 3Rs Centre. TCD-LBCAM has also successfully established a new *in vitro* NAM that may be used as an alternative to animal testing when studying the safety and efficacy of lung cancer therapies. Such an *in vitro* model, the ALI multi-layered culture (ALI MCC),[63,64] can replicate several of the Multidrug Resistance (MDR) and feedback activation loops seen in non-small cell lung cancer (NSCLC) patients. This research outcome has obtained official recognition by the EURL ECVAM as a promising advance in animal-free respiratory disease research (https://data.jrc.ec.europa.eu/dataset/). The LBCAM is member of the Cell Culture Criteria Panel, which has been created to discuss and formulate criteria for best practice in cell culture in preclinical cancer research. LBCAM is also expert member of the Animal Free Research Community of Practice and partner of the EC-funded REFINE project (Grant Agreement 761104), where its focus is set on the development of non-animal methods for the preclinical evaluation of the hepatotoxicity of nanobiomaterials. Its contribution to the field of scientific quality/translatability is documented in several peer-reviewed publications.[65–75]

Italy

The Italian *Centro 3R* uses its website (www.centro3r.it) and social media channels (Facebook: @Centro3R; Twitter: @Centro3; LinkedIn: https://www.linkedin.com/company/centro-3r), as well as workshops, media events, web events, annual meetings, researchers nights and seminars to disseminate their Three Rs content. The dissemination activities are funded by the member universities. In addition,

Centro 3R is often invited to share views via media debates. With regard to the core area of education on the Three Rs, Centro 3R members have set up a significant number of courses at different universities. For example, Centro 3R contributes to five Master's Degree courses and three PhD courses on the Three Rs principles, namely:

- At the University of Pisa, Master's Degree courses in the Faculty of Pharmacy (Prof. Daniela Monti), the Faculty of Chemistry and Pharmaceutical Technologies (Prof. Gabriella Ortore), the Faculty of Techniques in Animals Breeding and Cynophilist Education (Prof. Angelo Gazzano), and the Faculty of Biology (Prof. Michela Ori).
- At the Politecnico di Torino, a Master's Degree course in the Faculty of Biomedical Engineering (Prof. Valeria Chiono).
- At the Politecnico di Milano, a PhD course on 'Ethics in research' with Prof. Andrea Aliverti.
- At the Università degli studi di Milano-Bicocca, a PhD course on 'The principles of the Three Rs in biomedical studies' with Prof. Gabriella Nicolini.
- At the Università di Pavia, a PhD course on 'Scientific research and the Three Rs principles' with Prof. Livia Visai.
- At the Università di Genova, a PhD course on '3Rs principles for biomedical engineering' with Prof. Laura Pastorino.

Apart from these specific courses, teachers from Centro 3R educate on the Three Rs principles by giving a number of lectures as part of other Master's Degree courses, such as: 'Introductory course on animal experimentation', at the Mario Negri IRCCS Pharmacological Research Institute, Milan; '21st Century toxicology: Innovative models for risk prediction', at the Università di Genova; a course organised by Centro 3R for training of health service personnel (at CEFPAS in Caltanissetta), entitled 'Protection of animals used for scientific purposes. Research and innovation in the field of substitute methods'; and a lecture as part of the European Master of Translational Cosmetic and Dermatological Sciences, 'Nanoformulated cosmeceuticals: Skin permeability and toxicity', at the University of Piemonte Orientale, entitled 'Alternative methods for the validation of skin cosmetics, data obtained on 3D skin models' (Prof. Luisa Fiandra). Moreover, the Centro 3R has organised various workshops and training courses, such as the workshop 'Il principio delle 3R per una visione comune' [The 3R principles for a common vision] in October 2019 (Ministry of Heath, Rome) and the Advanced Theoretical Practical Course, 4th Edition 'Dare un senso ai metodi sostitutivi alla sperimentazione animale' [Making sense of alternative methods for animal testing] in November 2018 (Università di Genova), which was aimed at different professionals (surgeons, veterinarians, biologists, biotechnologists, chemists, pharmacists, biomedical laboratory technicians) and more recently a theoretical practical course on 'Innovative approaches in cosmetic testing, in compliance with European regulations', June 2022 (Università di Genova). Finally, with regard to research and scientific quality/translatability, Centro 3R encourages collaborative research through its website. Research outcomes are shared on the website as resources.

Luxembourg

The establishment of the Luxembourg 3Rs platform is currently in progress, and its website for the dissemination of Three Rs resources will be hosted on the website of the AEEC (Animal Experimentation Ethics Committee). LIST, together with ESTIV, organised a summer school in 2022 on *in vitro* methods. The development of courses at the University of Luxembourg (UL) for PI's and young researchers is in progress. In terms of Three Rs implementation, guidelines such as PREPARE, GCCP and GIVIMP will be included as much as possible in the review of projects by the internal ethics committee at the UL (AEEC). The implementation of *refinement* methods is part of the daily work with animals (e.g. tunnel handling, identification of improved enrichment material and refinement of surgical procedures). In the area of scientific quality/translatability, Luxembourg has a representative in PARERE at the JRC, and contributed to the development of two assays in the Thyroid Hormone Disruption Panel, which are currently under validation by the NETVAL network.

Netherlands

3Rs-Centre of the Utrecht University and University Medical Centre Utrecht: Several websites and databases are used for the dissemination of its Three Rs-relevant information, such as:

- its own website, https://www.uu.nl/3RsCentre;
- the 3Rs database programme, https://www.uu.nl/en/3rsdatabases, which is funded by different external sources and also includes the Humane Endpoints website (https://www.humane-endpoints.info/en);
- the Interspecies database, https://www.interspeciesinfo.com/; and
- the FCS-free database and information site, https://fcs-free.org/

Websites with information on education and training in laboratory animal science (LAS) include:

- for general information;
- for LAS e-learning modules, https://www.uu.nl/en/organisation/3rs-centre/education-and-training/e-learning-modules;

— for codes of practice;
— for Three Rs search methods, https://www.uu.nl/en/organisation/3rs-centre/3rs/3rs-search-methods

In addition, the website https://www.uu.nl/organisatie/3rs-centre/3vs/scholieren presents information and papers for high school students (only in Dutch) and the website https://elbd.sites.uu.nl/2017/04/21/update-dieren-en-dierproeven-in-het-onderwijs/ provides information for high school teachers on animal experiments and non-animal methods for use in their teaching (only in Dutch).

Moreover, the centre publishes a bimonthly newsletter, runs a Twitter account (@3VCentrumULS) and is involved in funding of local Three Rs projects in co-operation with the Animal Welfare Body-Utrecht. In addition, the centre is involved in the Laboratory Animal Science Course (FELASA accredited), an Advanced *In Vitro* Methods Summer School (at Utrecht University), and acts as advisor to the European drive to validate *in vitro* methods for the detection of thyroid disruptors (https://ec.europa.eu/jrc/en/science-update/vitro-methods-detection-thyroid-disruptors). In the area of scientific quality/translatability, the centre raises awareness on good *in vitro* methods — in particular, it focuses on the issues related to the use of fetal bovine serum and stimulates the use of xeno-free cell and tissue culture media.

NCad: NCad is a partner of the TPI (Transition Programme for Innovation without the use of animals) in the Netherlands. The approach of the TPI is: *What is the research question, and how can we get the best answer, preferably without animals?* It is not: *Is this animal-free method good enough to replace an animal experiment?* That is why this transition movement works with interdisciplinary networks of stakeholders that form the whole chain. The TPI tries novel approaches to find new methods and to increase their acceptance and use, not only in innovative research that helps patients, but also in making better safety predictions for products that contain chemical substances of concern, for the benefit of employees and consumers. TPI partners are co-operating on the ambition of 'the Netherlands as a frontrunner in the international transition of innovation without the use of animals'. The mission is to develop alternative models and tests that better predict the effectiveness and safety of medicines and substances, thus making animal procedures increasingly redundant. The Ministry of Agriculture, Nature and Food Quality directs the TPI on behalf of the other ministries involved. The directors and staff of the Ministries involved hold interdepartmental consultations, via the Dutch Interdepartmental Working Group on Alternatives to Animal Testing (D)IWAD, on alternatives to animal procedures. TPI partners have set up a guiding Core Group and an action-coordinating Transition Group for TPI. Detailed information about the partners of the TPI can be found at: https://www.animalfreeinnovationtpi.nl/. As part of the TPI, NCad has already published numerous advisory reports, such as:

— *Genetically modified animals. Died or killed before use in breeding programmes or animal procedures: Part 1 and 2 Quality criteria;*
— *Motivation by restriction? Starting points for controlled fluid and food intake in neurocognitive research from a 3Rs perspective;*
— *Transition to non-animal research: About the possibilities for phasing out animal procedures and stimulating innovation without laboratory animals;*
— *Rehoming of former laboratory animals; Alternative methods for killing laboratory animals; Preventing, recognising and combating pain in laboratory animals;*
— *Synthesis of evidence in laboratory animal research;*
— *Indicators, management and utilisation of data for monitoring laboratory animal use and 3R alternatives: Part 1 and 2;*
— *Procedures using cats or dogs; and*
— *Target images on animal-free research.*

With regard to the last report listed above, '*Target images on animal-free research*' describes clear transition objectives for each research domain, aimed at reducing the use of laboratory animals with equal or better quality research methods. This requires a different way of thinking and working, and abandoning animal testing as the 'gold standard'. By creating and realising an ambitious target, each research domain takes its responsibility to accelerate the transition to animal-free research. At the moment, experts are working on target images in the cardiovascular domain, in the immunological and educational domains.

NCad is also currently working on: the use of human tissue in research; COVID-19; parallel studies; behavioural research; a view on cumulative discomfort; and animal models that we want to stop using. A more complete account of the activities of NCad is available at https://english.ncadierproevenbeleid.nl/. The areas in which NCad is working, in collaboration with its TPI partners are:

— sharing practices and facilitating dialogue between professionals (Ministry of Agriculture, Nature and Food Quality);
— programme-coordinated research: 'Securing innovation without animal testing' (ZonMw), 'Research programme with human measurement models' (association of Dutch Health foundations, with dsRAT and others), and 'Combining *in vitro* and, for example, AI' (consortium of dsRAT, TNO and Galapagos);
— the building of a new safety assessment system, carrying out work packages in the VHP4Safety project;

— coordinating the VHP4Safety project, with an inter-departmental call in the National Science Programme and with the EU-partnership PARC (RIVM);
— drawing up target images in various fields (NCad), with 'Targets for education' (NFU and VSNU);
— developing tools that help accelerate the transition to non-animal methods, with 'Pay attention to the evaluation and registration of research and guaranteeing expertise in systematic literature analysis' (ZonMw), 'Beyond Animal Testing Index deploy' (see www.beyondanimaltesting.org) and 'Using vital tissue (see www.vitaltissue.nl).

Norway

Norecopa's website aims to be a global one-stop-shop for quality Three Rs resources. The website currently contains approximately 8900 pages, with resources of relevance to all the Three Rs. In addition, Norecopa issues a detailed newsletter seven to eight times a year. Norecopa hosts the website of the International Culture of Care Network (https://norecopa.no/coc), and maintains an interactive map of global 3R centres, networks and organisations (https://norecopa.no/global3R). Norecopa also runs a closed discussion forum for key personnel at Norway's animal research facilities, where they can discuss issues of general interest. Norecopa contributes funding to research projects on a case-by-case basis, and its Secretary advises the Norwegian regulatory authorities and the National Committee for the protection of animals used in research. Within the educational sector, Norecopa's website contains links to information about animal research for schools,[76] teaching aids for educators,[77] a webpage with links to resources[78] about ethics, and links to courses from all over the world in Laboratory Animal Science. Moreover, Norecopa has arranged a large number of international meetings[79] and published the PREPARE[13] guidelines for planning animal studies (https://norecopa.no/PREPARE) in collaboration with British and Norwegian scientists. PREPARE can contribute significantly to the fields of scientific quality and translatability. Finally, Norecopa's Secretary also lectures regularly on planning animal studies (39 presentations in 17 countries in 2021).

Poland

As part of its activities, the *CMA* published the free-of-charge quarterly official journal, *Vitryna* (1999–2002), which described and disseminated information on alternative methods. A CD-ROM 'Training material for toxicity assessment with alternative methods', including a tutorial on the most important *in vitro* methods (at that time) was attached to one of the *Vitryna* issues in 2001. Its work on the implementation of the Three Rs included the establishing of GLP standards at the CMA laboratory in 2008, mainly for studies performed for regulatory purposes, i.e. cytotoxicity (the MTT and NRU assays), genotoxicity (the mouse lymphoma assay, the *in vitro* micronucleus test). In addition, cell culture methods are also carried out in compliance with GCCP and the principles of GIVIMP. Recently, an *in vitro* method for the assessment of skin irritation by using a reconstructed human epidermis model (also in accordance with GLP standards) has been added to the portfolio of GLP tests performed at NIOM. With regard to the core area scientific quality/translatability, the CMA at NIOM acts as one of the NETVAL laboratory members and was involved in a prevalidation study that aimed to implement/establish a new method for the detection of potential thyroid disruptors (the T-screen by using GH3 cell line). Unfortunately, after finishing Part 1 of the prevalidation study, the current financial and personnel situation forced the CMA to suspend further research in this project. In 2011–2012, an attempt was made to implement the new *in vitro* method for the assessment of carcinogenicity, i.e. the *in vitro* cell transformation assay (CTA; by using Balb/3T3 clone A31-1-1 and following recommendations described by EURL ECVAM[80] and Sasaki et al.[81]). The method was implemented, but no more studies were performed. In 2012–2013, scientists from the Molecular Toxicology Laboratory at NIOM/CMA participated in a validation study on 'Colony forming efficiency assay', sponsored by the JRC.[82] Presently, the main activity of the CMA is connected with the new TWINALT project, coordinated previously by Dr Maciej Stępnik and currently by Prof. Edyta Reszka, in partnership with VUB, NILU and UMIL. During the project, NIOM aims to increase its capacity (portfolio) on available *in vitro* methods for toxicity testing. The main goals of the project are: to exchange scientific knowledge on alternative methods by twinning with all partnering institutions in training strategies (e.g. Science and Technology courses and training in transferable skills); to communicate with society; and to disseminate information on alternative methods to toxicity assessment as widely as possible.

Portugal

The Three Rs centre at *i3S* (Portugal) is being established, and will have its own website and social media presence. Currently, it disseminates information through the REMODEL project, as well as the i3S institutional website and social media accounts. Its stakeholder engagement currently runs primarily through HealthCluster Portugal, its outreach activities focus on students (high school and university), and its social media communication has a core message related to animal research and laboratory animal care. The centre's educational activities are comprised of FELASA-accredited training for Functions A, B and D, training

courses in experimental design and statistics for animal experiments, training courses in organoids as biomedical research models, and a summer school for high school students on animal welfare, including the Three Rs. These educational activities are aligned with the doctoral programmes at the institution, to which the centre also contributes with ethics lectures. Its efforts in scientific quality/translatability include work on animal research ethics, *refinement* and laboratory animal welfare, as well as on *replacement* through the use of organoids and other advanced Three R models. The centre also supports researchers using different models through the various i3S scientific platforms,[83] such as 'Animal Facility', 'Cell Culture and Genotyping' and '*In vivo* CAM Assays'.

Slovakia

Some details of the activities of *SNP3Rs* have already been described above. Its focus is on training, validation activities and consulting work.

Spain

CMCiB runs a Three Rs seminar programme for the research community, and educates society through visits to the centre. The implementation of new approaches is accomplished by including mathematical models in preclinical research. Relevant aspects of *refinement* and animal welfare, *reduction* through the use of bioimaging, and *replacement* through the promotion of alternatives such as organoids, *Drosophila* and zebrafish in translational research, are all important topics at CMCiB.

Sweden

As the executive body of the National Committee, the *Swedish 3Rs Center* carries out the majority of the Committee's assignments. The centre also promotes animal welfare and continuously works to reduce the number of animals used in experiments and to replace the animals still used with animal-free methods whenever possible. The Swedish 3Rs Center collects, compiles and actively disseminates information on the Three Rs, provides advice and recommendations on the topic, supports competent authorities in their strategic work involving the Three Rs, promotes the collaborative use of organs and tissues, and develops, assesses and shares best practices regarding animal use for scientific purposes.

The 3Rs Center mainly disseminates information through its website (http://jordbruksverket.se/3R), social media (LinkedIn and Twitter) and email lists. The centre produces articles, news updates and a digital newsletter called *Focus on the 3Rs*. It also organises workshops and seminars on different topics, sometimes in Swedish and sometimes in English. The centre hosts an annual meeting and local Three Rs days for animal welfare bodies, and works together with other Swedish government agencies on Three Rs issues. The centre plays a part in education in several different ways. It organises *refinement* workshops at research animal facilities, and educational activities for the animal ethics committees. It has produced digital educational material and information graphics aimed at high school students, as well short films focused on the replacement of animal models. In addition, it also meets high school students at the science festival 'European Researchers' Night'. One of the centre's main tasks is to provide advice and recommendations on the Three Rs. So far, it has focused on the group-housing of male mice, and the marking and tagging of fish. These recommendations are available on the centre's website (in both Swedish and English), as a report and as posters to print. Another of its tasks is to facilitate communication on the Three Rs. Therefore, the centre hosts the Swedish 3Rs Network for Replacement — a national network and specific LinkedIn group for discussions on *replacement*.

Switzerland

The Swiss *3RCC* has set its focus on the following areas: research; research and monitoring; and education and communication. Since its inception in 2018, the 3RCC has provided CHF 1.26 million each year, in research funding for scientifically sound projects with a Three Rs impact. The Open Call funding scheme, developed at the outset, has proven useful in attracting a broad range of applications across all major research fields in the life sciences.

For its 2020 call, the 3RCC received 69 outline applications. Half of the projects related to *replacement*, 30% to *refinement*, and 20% to *reduction*. The 3RCC selected five high-impact projects, to be financed on a total of CHF 1.4 million. Two projects are in the field of neurology and one in cancer; these are two areas of research in which high numbers of laboratory animals are being used in Switzerland. To increase the visibility of Three Rs researchers and their achievements, the 3RCC offers annual awards to scientists who have significantly contributed to the advancement of the Three Rs in the life sciences in Switzerland.

Integral to the work of the 3RCC is its internal project portfolio, which includes various surveys to monitor the progress in Three Rs implementation in the areas of education and research. Early in 2021, two reports were published by the 3RCC — one on the use of non-aversive handling methods, and the other on the extent of animal use in Swiss science; these will serve as a basis for future fact sheets. An additional report on the use of animals in Swiss education is planned for release shortly. The 3RCC takes an active role in supporting the coordination and mapping of

existing educational activities, covering the Three Rs at Bachelor degree level. The development of targeted training in the area of *refinement* was a focus in 2020 and 2021. The execution of these activities was impacted by COVID-19 restrictions, and in response, virtual formats were developed to address educational needs on a remote basis. The 3RCC is committed to active communication with its stakeholders. In December 2020, the first steps were taken to form a Communications Work Group, in which communications experts of the internal stakeholders are represented. The mandate of the group in 2021 is the development of a communications strategy, with a focus on future public outreach efforts of the 3RCC. During 2020, the 3RCC expanded its international network through its contribution to the Pan-European 3Rs Day, which was held virtually.

United Kingdom

In the UK, the *NC3Rs* has a strong focus on Three Rs communication and dissemination. The NC3Rs website (https://www.nc3rs.org.uk/) received 729,000 page views from 236 countries in 2021. Its newsletter goes to over 6400 users (and more via distribution through various networks) and, in recent years, the organisation has made extensive use of social media (https://twitter.com/NC3Rs) to increase visibility. The NC3Rs has hosted a large number of free-to-attend workshops and symposia in the UK and other countries, to share outcomes from its activities and encourages (through small grants) NC3Rs grant holders to participate in public engagement activities.

It commits approximately £8 million per annum for research and innovation, but this varies year on year. This is divided into research grants and CRACK IT. The former are response mode grants for projects in all three 'Rs', with the majority (65%) for *replacement* research. NC3Rs schemes have attracted co-funding from other research funding bodies for projects in specific areas of need. Funding has also been allocated for infrastructure projects, and skills and knowledge transfer awards (the latter to tackle the lag between development and uptake of Three Rs approaches). To aid dissemination and uptake of new methods arising from NC3Rs-funded research, a dedicated publishing platform (the NC3Rs gateway) for detailed methodologies and articulation of Three Rs impacts has been launched in partnership with F1000Research.[84]

CRACK IT Challenges (https://www.nc3rs.org.uk/crackit/) is an original innovation programme that supports the translation of scientific discoveries into products and services with a Three Rs impact. CRACK IT Challenges are identified typically by industry, who then partner with the NC3Rs to create a Challenge brief to be solved by the academic/SME community in the form of a multidisciplinary partnership. The Challenges directly address major issues facing industry and the bioscience sector, and while the NC3Rs funds the research, the industry partners commit considerable resources in-kind to advance the project. CRACK IT succeeds because all stakeholders benefit — the NC3Rs benefit from Three Rs advances, industry sponsors have their problems addressed, and the academic/SME consortia benefit from validation of their technologies and introduction to new customers.

With regard to education and training, in the last five years, the NC3Rs has funded nearly 100 early career training awards, either as PhD studentships or postdoctoral training fellowships. The NC3Rs also puts much effort into changing policy, practice and regulation. Many of the NC3Rs staff lead programmes aimed at organisations that influence the use of animals in research — either by funding it, conducting it, requiring it, licensing it, or publicising the findings from it. These in-house programmes address Three Rs opportunities that cannot be delivered through research funding and are focused on four areas: improving the experimental design, analysis and reporting of *in vivo* and *in vitro* research; working with research funders and universities; embedding the Three Rs in pharmaceutical and chemical safety regulations; and championing animal welfare.

For the first of these areas, the Experimental Design Assistant (EDA; https://eda.nc3rs.org.uk/) is a unique online system that helps researchers design robust *in vivo* experiments. Many major organisations, both in the UK and internationally, now recommend use of the EDA to grant applicants. The ARRIVE guidelines (https://arriveguidelines.org) for reporting animal research are the international standard endorsed by over 1000 journals, and are included in recommendations from national and international funders (e.g. the US National Institutes of Health). Recently, the guidelines have been revised to improve clarity and encourage more widespread adoption. The NC3Rs plans to create related guidelines for the design, analysis and reporting of *in vitro* research (the RIVER project), and some preliminary work has already been done to facilitate this.

For the second area, the NC3Rs is working directly with universities in the UK to encourage their commitment to high standards of animal work. The principal instrument is the appointment of Regional Programme Managers, jointly funded by the NC3Rs and the universities involved, who work directly with researchers and animal technicians to promote better implementation of the Three Rs. The NC3Rs also provides a peer-review service (https://nc3rs.org.uk/peer-review-and-advice-service) for major funders internationally, with a particular focus on applications involving the use of sensitive species (such as non-human primates).

In the third area, the NC3Rs has a large programme of work aiming to reduce reliance on animal testing for drug and chemical safety (https://www.nc3rs.org.uk/3rs-toxicology-and-regulatory-sciences). This has involved establishing a track record of trust with regulators and companies, which has

facilitated transparency and data sharing initiatives. In the last five years, there have been 17 individual projects involving in-house collation and analysis of data from 58 companies, to examine the evidence base for existing safety tests and to identify opportunities for the Three Rs. These projects have typically involved regulatory authorities from the UK and other countries. This model of working has produced major successes working within current regulations (e.g. reducing use of recovery animals), as well leading to changes in regulation (e.g. removal of acute toxicity testing; https://www.nc3rs.org.uk/single-dose-acute-toxicity-studies) from the ICH M3 guidelines, and adoption of the fixed concentration procedure, OECD TG 433, (https://www.nc3rs.org.uk/adoption-fixed-concentration-procedure-acute-inhalation-studies) for inhalation toxicity.

Finally, in the fourth area, the in-house programme on animal welfare is an essential part of the NC3Rs *refinement* strategy (which has been published in *Lab Animal*).[85] The programme has had national and international impacts benefitting the welfare of potentially millions of animals. It is unique in its scope and approach, focusing on minimising pain, suffering and distress, promoting positive welfare states, and highlighting the associated scientific advantages of improving animal welfare. More recent work on rodent welfare has focused on refining models of disease (e.g. stroke,[86] epilepsy[87]), reducing aggression in group-housed male mice[88] and translating research into practice (e.g. mouse handling,[89] euthanasia). The programme on non-human primate welfare (https://www.nc3rs.org.uk/welfare-non-human-primates) is the largest in the world, and has had major influence on housing, methods of restraint and behavioural management.

Ukraine

The *Ukrainian 3Rs Center* collaborates with the national parliament and government. Part of this work resulted in a new Regulation of Cosmetic Products in the Ukraine, where the testing of cosmetics on animals is now forbidden. Collaboration with the Pesticides Registration Commission in the Ukraine led to reports on alternative tests that are accepted for consideration and obliged the industry to prioritise alternative tests. The centre also distributes Three Rs knowledge and implements the Three Rs in education. For example, the module 'Alternative methods, Three Rs principles' was included in the Toxicological Course at The National University of Food Technologies, where the participants are future technologists of cosmetics products. The module is also a part of a Toxicology Course for experts (people from the government, industry, regulators, scientists). Moreover, a video clip about alternative methods was broadcast on one of the national channels in the Ukraine. The Ukraine 3Rs Center has already implemented several alternative methods in its laboratories, such as three *in vitro* methods (OECD TG 431, OECD TG 439, OECD TG 492) at the facilities of L.I. Medved's Research Center of Preventive Toxicology, Food and Chemical Safety, Ministry of Health. This large research centre is the principal Toxicological Research Center in the Ukraine, and as such, these activities will help save the lives of many animals.

Summary

This second article in the series describes the current status and activities of European Three Rs centres and platforms. The institutions that contributed to this article summarised their individual structures, core areas and tasks. This 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 various Three Rs institutes are very diverse, with the 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.

A third article in the series will focus not only on the future projects and plans of the Three Rs centres and platforms, but also on their future needs and opportunities for improvement of the Three Rs institutions. 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.

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Appendix. The acronyms and abbreviations mentioned in the article.

Acronym/abbreviations	Explanation
3RCC	Swiss 3R Competence Centre
ALI	Air–liquid interface
AOP	Adverse Outcome Pathway
APVV	Slovak Research and Development Agency
ARRIVE	Animal Research Reporting *In Vivo* Experiments — Guideline
AWB	Animal Welfare Body
AWON	Animal Welfare Officers Network
BB3R	Berlin-Brandenburg Research Platform
Bf3R	German Centre for the Protection of Laboratory Animals
BfR	German Federal Institute for Risk Assessment
BMBF	Federal Ministry of Education and Research (Germany)
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
CHO	Chinese hamster ovary cells
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)
CSD	Scientific Teaching Council (Consiglio Scientifico Didattico)
CSL	Clinical skills laboratory
CTA	Cell transformation assay
DAAD	German Academic Exchange Service (Deutscher Akademischer Austauschdienst)
DFG	German Research Foundation
(D)IWAD	Dutch Interdepartmental Working Group on Alternatives to Animal Testing
DK-EPA	Danish Environmental Protection Agency
DNT	Developmental neurotoxicity
DNT-IVB	Developmental neurotoxicity *in vitro* battery
dsRAT	Dutch Society for the Replacement of Animal Testing
EC3R	Einstein Center 3R
ECHA	European Chemicals Agency
EDA	Experimental Design Assistant
EFSA	European Food and Safety Authority
EPAA	European Partnership for Alternative Approaches to Animal Testing
EPFL	Ecole Polytechnique Fédérale de Lausanne
ESTIV	European Society for Toxicology *In Vitro*
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
EUSAAT	European Society for Alternatives to Animal Testing
FAIR	Findable, Accessible, Interoperable and Reusable concept for research data
FELASA	Federation of European Laboratory Animal Science Associations
FHNW	University of Applied Sciences and Arts Northwestern Switzerland
FOPH	Swiss Federal Office of Public Health
FSVO	Swiss Federal Food Safety and Veterinary Office
FTE	Full-time equivalent
FU Berlin	Freie Universität Berlin (Germany)

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Acronym/abbreviations	Explanation
GCCP	Good Cell Culture Practice
GIVIMP	Guidance Document on Good *In Vitro* Method Practices
GKF	Society for the Promotion of Dog Research (Gesellschaft zur Förderung Kynologischer Forschung e.V.)
GLP	Good Laboratory Practice
GPAP	Good Read-Across Practice
hiPSC	Human induced pluripotent stem cell
HU Berlin	Humboldt University Berlin
i3s	Institute for Research & Innovation in Health (Instituto de Investigação e Inovação em Saúde da Universidade do Porto)
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)
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
Icopa	Irish consensus platform for alternatives
IEFT CEM	Institute of Experimental Pharmacology and Toxicology, Center of Experimental Medicine
IGTP	Germans Trias i Pujol Research Institute
ISO	International Organisation for Standardisation
IUF	*Leibniz Institut für umweltmedizinische Forschung* (Leibniz Research Institute for Environmental Medicine), in Düsseldorf (Germany)
IVTD	*In Vitro* Toxicology and Dermato-Cosmetology (at Brussels Health Campus, Belgium)
JRC	European Commission Joint Research Centre
LAS	Laboratory Animal Science
LBCAM	Laboratory for Biological Characterisation of Advanced Materials
LIH	Luxembourg Institute of Health
LIST	Luxembourg Institute of Science and Technology
LTK	Institute of Laboratory Animal Sciences in Switzerland (Institut fur Labortierkunde)
MARD SR	Ministry of Agriculture and Rural Development (Slovak Republic)
MDC	Max-Delbrück Centre in Berlin, Germany
MDR	Multidrug Resistance
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide
MWK	*Niedersächsisches Ministerium für Wissenschaft und Kultur* (Ministry of Science and Culture of Lower Saxony)
NAM	New approach methodology
NASH	Non-alcoholic steatohepatitis
NC3Rs	The National Centre for the 3Rs (UK)
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
NILU	Norwegian Institute for Air Research
NIOM	Nofer Institute of Occupational Medicine in Lodz (Poland)
NIPH	National Institute of Public Health (Czech Republic)
Norecopa	National Consensus Platform for Alternatives in Norway
NSCLS	Non-small cell lung cancer
NRU	Neutral Red Uptake (assay)
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
PROKOS	Association of producers, importers and distributors of cosmetics and their ingredients (Czech Republic)
R2N	Initiative 'Replace and Reduce from Lower Saxony' (Germany)
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RepRefRed Society	Gesellschaft zur Förderung von Alternativen Biomodellen (Society which aims to promote the Three Rs; Austria)
RESAL	*Réseau des animaleries lémaniques* (Lemanic Animal Facility Network in Switzerland)
RIVM	National Institute for Public Health and the Environment (Netherlands)

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Acronym/abbreviations	Explanation
RKI	Robert Koch Institute (Berlin, Gemany)
ROCAM	Romanian Centre for Alternative Test Methods
RWTH Aachen	*Rheinisch-Westfälische Technische Hochschule Aachen* (RWTH Aachen University)
SAFN	Swiss Animal Facilities Network
SAP	Swiss Animal Protection (SAP) organisation
SAS	Slovak Academy of Sciences
SCCS	Scientific Committee on Consumer Safety (EC)
SET	*Stiftung zur Förderung der Erforschung von Ersatz – und Ergänzungsmethoden zur Einschränkung von Tierversuchen* (SET foundation, Germany)
SETOX	Slovak Toxicology Society
SERI	Swiss State Secretariat for Education, Research and Innovation
SNP3Rs	Slovak National Platform for 3Rs
TARCforce 3R	Research Center for Animal Welfare and Laboratory Animal Science (Germany)
TCD	Trinity College Dublin (Ireland)
TCD-CMU	Trinity College Comparative Medicine Unit (Ireland)
TNO	Netherlands Organisation for Applied Scientific Research
TPI	Transition Programme for Innovation (Netherlands)
TSH	Thyroid stimulating hormone
TU Berlin	Technische Universität Berlin (Germany)
UL	University of Luxembourg
UMIL	*Università degli Studi di Milano* (University of Milan, Italy)
US-EPA	US-Environmental Protection Agency
USI	*Università della Svizzera Italiana* (University of Italian Switzerland, Lugano)
US-NTP	US-National Toxicology Program
UZH	University of Zurich (Switzerland)
VEGA	*Vedecká grantová agentúra* (Scientific grant agency, Slovak Republic)
VUB	Vrije Universiteit Brussel (Belgium)
VZET	*Virtuelles Zentrum für Ersatz- und Ergänzungsmethoden zum Tierversuch* (Virtual Centre for Alternative and Complementary Methods to Animal Testing), University of Veterinary Medicine, Hannover (Germany)
ZHAW	Zurich University of Applied Sciences
ZonMw	Netherlands Organisation for Health Research and Development