European Commission

Towards replacement of animals for scientific purposes

SCIENTIFIC CONFERENCE REPORT

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#NonAnimalScience

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Executive Summary

Towards replacement of animals for scientific purposes

The Scientific Conference "Towards replacement of animals for scientific purposes" took place online on 2 and 3 February 2021. It was organised by the European Commission, with the aim of accelerating the move away from using animals in scientific research and regulatory testing.

The main objectives of the virtual conference were to:

- *Provide an update on the Commission's work towards the ultimate goal of replacing animals in science by advancing non-animal alternatives;*
- *Demonstrate how education, training, and increased transparency on animal use, can help speed up the transition to non-animal approaches;*
- *Showcase the most recent scientific advances in non-animal methods.*

The conference was attended by a diverse audience, with well over a thousand people registering from all around the EU and beyond. Knowledge was shared among people from universities and research institutes, NGOs, regulatory bodies, and policymakers at EU and Member State level.

This is the second conference that the European Commission has organised on this topic; the first took place in Brussels in December 2016.

Virginijus Sinkevičius (European Commissioner for the Environment, Oceans and Fisheries) opened the 2021 conference by outlining the Commission's ambition on this topic. He stressed that the goal is to only use animals in science when it is absolutely necessary to save lives and protect the environment, and where alternatives are not yet available.

The main policy instrument is Directive 2010/63/EU on the protection of animals used for scientific purposes. The Directive is based on the Three Rs principle: to Replace, Reduce and Refine the use of live animals.

The conference presentations and discussions were structured within four sessions, on transparency; education and training; cutting-edge science; and gaining trust in using new alternative approaches.

In his closing remarks, **Maurice Whelan** (Joint Research Centre) said the multi-faceted conference programme and the diverse backgrounds of participants reflected the cross-cutting nature of the Three Rs. He noted the healthy discussions and the large degree of consensus which are important to make progress together on many fronts. There is growing willingness to mainstream non-animals methods, he said, and now we need the belief to make it happen.

How transparency can accelerate transition to non-animal science

The first session focused on the importance of transparency in mainstreaming a shift towards non-animal methods. In 2020, the publication of two European Commission reports, on the implementation of Directive 2010/63/EU and on animal use statistics, made the EU a world leader in terms of transparency in the use of animals for scientific purposes.

Susanna Louhimies (DG Environment) and **Pierre Deceuninck** (JRC) explained how the Commission is making available animal use data, which has been collected as an obligation under Directive 2010/63/EU. The first reports in 2020 summarise data for 2015-2017. However, the Commission is committed to taking transparency even further, which is clearly demonstrated by the 2019 amendment to the Directive.

As a result, they gave a 'sneak peek' of a new easily-searchable public statistical database called ALURES. At its launch, towards the end of March 2021 and two years ahead of schedule, it will contain EU-level statistics, with Member State-level data available from 2023. The presentation also focused on Non-Technical Summaries of authorised projects that Member States are required to produce under the Directive, which will soon also be accessible through another Open Access public database.

Jean-François Dechamp (DG RTD) described how the Commission is promoting Open Science. It will play an increasingly important role under the new Horizon Europe programme. The European Commission open access publishing platform, Open Research Europe (ORE), for instance, will provide Horizon 2020 and Horizon Europe grant beneficiaries with a free-of-charge, high-quality publishing service.

Serban Morosan (League of European Research Universities) presented the findings of a LERU report (December 2020) on good practice in communicating animal research at universities. Transparency agreements have been signed by participating universities, committing them to be more open about animal research. Transparency is essential for developing trust, and is the basis for public support for continued work on animals until they can be replaced by alternatives, he said.

Zoltan Dienes (University of Sussex, UK) explained the benefits of registered reports, an increasingly important area of publishing. Scientists send their proposed research methodology to journals. If this is accepted by peer-review then the paper is guaranteed publication, whatever the results. This addresses a bias in the scientific literature, which is skewed toward positive results. In the context of animal research, all the data is published transparently, genuinely valuable and reproducible, he said.

The moderated discussion that closed the session included audience questions. For example, these addressed the possible limits of transparency and Open Science, such as confidentiality and privacy; and the importance of publishing negative results, to avoid the duplication of work and make data reusable. The EU is a pioneer in the field of transparency, concluded Ms Louhimies, and the ambition is now to expand this to the global level.

Session 2:

Education & Training with focus on non-animal approaches and implementation of the Directive

This session focused on education and training, a legal requirement under Directive 2010/63/EU, and highlighted a broad range of teaching tools that aim to accelerate the move to non-animal approaches. Five presentations were followed by three parallel breakout groups, where participants could experience some of these tools.

Katrin Schutte (DG Environment) talked about the E-modules developed as part of the Education & Training Platform for Laboratory Animal Science (ETPLAS). The first four concern Three R elements (e.g. design of procedures and projects), while two new E-modules are being developed on non-animal alternatives. During the lunchbreaks on both days, participants could view the first four E-modules.

Daniela Salvatori (Utrecht University) described how plastinated models and Virtual Reality are replacing live animals in the curriculum of the Veterinary Faculty at Utrecht University. Durable soft and hard plastinated cadavers, for example, are used for teaching anatomy and training for clinical procedures. These approaches have reduced the use of animals by at least 60%, she said.

Julia Malinowska (University of Birmingham, UK) related her experience, as a PhD student, of taking part in the JRC Summer School on non-animal approaches in science. She described the content of the intensive four-day course in Ispra, Italy, and how it helped her career development related to the Three Rs.

Marcelle Holloway (JRC) described how the JRC is promoting the Three R concept in education programmes. She introduced the JRC report 'Introducing the Three Rs into secondary schools, universities and continuing education programmes', and a MOOC (Massive Open Online Course) aimed at Life Science teachers.

Lindsay Marshall (Humane Society International) described an educational course on New Approach Methods (NAMs) being developed by Humane Society International. It will enable researchers to look at the range of non-animal tools available, and provide easy-to-understand information for the general public.

The first of the three breakout groups, led by European Schoolnet, looked at the JRC MOOC for high schools. Conference participants played the role of 14-year old students to gain insights into how the MOOC works in high schools.

In the second breakout group, participants took part in a mentimeter survey, which is a tool used in the Toxicity Master's programme at Karolinska Institute in Sweden. This included rating methods they thought contributed most to reducing animal use.

The third breakout group involved an interactive learning module that will be used in an upcoming JRC Summer School; an 'escape room' scenario where participants had to construct an adverse outcome pathway (AOP).

Audience questions included ones on the right age to introduce this issue in education; and the extent to which animals can now be fully replaced in different areas of teaching, with speakers contrasting biomedical research to veterinary training. It depends on the type of skills being taught, concluded Dr Schutte.

Cutting-edge science: latest scientific advances to improve research and testing tool box

This session provided an overview of the recent models and approaches that are being employed to address research needs that previously relied on animal models.

Ans Punt (Wageningen Food Safety Research) talked about computer models, called physiologically based kinetic (PBK) models. These predict the behaviour of chemicals *in-vivo* (whole organism), which are used for a better interpretation of *in-vitro* toxicity data. This extrapolation step is crucial in non-animal testing strategies, though standardisation is needed to gain regulatory confidence in the model predictions, she concluded.

Maddalena Fratelli (Mario Negri Institute) explained how *in-silico* methods and data reuse can reduce animal use. Machine learning and 'omics data (e.g. genomics) can predict drug sensitivity, for example, to select patients who will most benefit from treatments. There is a big opportunity in personalised medicine where animal models are lacking, she said.

Peter Loskill (Eberhard Karls University Tübingen) talked about developments in Organ-on-Chip technology. These now incorporate a range of human tissue (e.g. eye, heart, and lung). Their use has been mainly for drug development, but they could have many applications. He introduced an enabling technology, the Organ-Disc, which makes the technology scalable and easier to use.

Giel Hendriks (Toxys) described ReproTracker, an *in-vitro* assay for developmental toxicology. For the assay, pluripotent human stem cells are differentiated into the different embryonic tissue types. Biomarker genes are used to show the degree to which chemicals disrupt developmental processes. Using well-known reprotoxic compounds the predictivity of the assay is quite remarkable, he said.

Christodoulos Xinaris (Mario Negri Institute) talked about engineering patient-specific tissue *in-vitro*. Patient-derived 3D tissues are being used for studying human development, disease modelling and drug testing. The use of this technology to test drugs against polycystic kidney disease has significantly reduced animal use. His team also engineers human tissues for studying the diabetic heart and kidney, and for regenerative medicine applications.

Francesca Pistollato (JRC) and **Laura Gribaldo** (JRC) introduced the JRC's reviews of advanced non-animal uses in biomedical research. The first two, on respiratory tract diseases and breast cancer, have been published, and five more are to come. Moreover, they presented recent JRC activities aimed at defining suitable indicators to retrospectively monitor impact and innovation of EU-funded biomedical research, particularly for Alzheimer's disease and other dementias, breast cancer and prostate cancer. They then interviewed three scientists who work on non-animal models: **Erwin Roggen** (ToxGenSolutions) on Alzheimer's disease, **Anne van der Does** (Leiden University Medical Center) on lung disease, and **Joan Montero** (Institute for Bioengineering of Catalonia) on cancer.

The moderated discussion addressed several topics, such as the importance of collaboration, for example between scientists and risk assessors, the concept of toolboxes and integrated approaches that combine different non-animal models to replace animal methods (not 1-for-1). As another key takeaway, it was recognised that the use of patient-derived stem cells holds promise in the field of personalised medicine, ensuring human relevance and enabling high throughput applications.

Session 4:

Gaining trust in using new alternative approaches

The last session showcased how organisations across different sectors are gaining confidence in using the new non-animal approaches.

Ard Teisman (Janssen Pharmaceuticals) presented an industry point-of-view of organ-on-chip technology. He focused on the drivers that are leading pharmaceutical companies to implement stem cell *in-vitro* models and introduced the role of such models in early safety pharmacology testing.

Shahjahan Shaid (GSK) described the Vac2Vac project, which aims to improve batch-to-batch consistency of human and veterinary vaccines using non-animal methods. The range of new quality control assays being tested and validated for human and animal diseases could replace assays using a range different animals.

Marcel Leist (University Konstanz) used developmental neurotoxicity as example area to show that it is important for test developers to also devote energy to validation of their tests. He presented examples from the EU-ToxRisk project on how different measures can be taken to increase regulators' trust in non-animal methods.

Carl Westmoreland (Unilever) presented case studies for assuring safety without animal testing. For example, his team imagined no data existed for coumarin, an ingredient in personal care products; they used non-animal methods to prove its safety. Unilever launched a new surfactant in a washing up liquid in Chile, using only non-animal Next Generation Risk Assessment (NGRA).

Rhiannon David (AstraZeneca) talked about the use of organ-on-chip models to replace animals in drug development, especially for safety assessment where they better mimic human pathology. She described how a bone marrow-on-chip platform is being used to optimise the drug doses and scheduling in the clinic, to reduce toxicity due to drug interactions.

Dilyana Filipova (European Coalition to End Animal Experiments) described a range of non-animal methods that are being used in COVID-19 research. These include lung, brain, small intestine, and lymph node organoids. They have the advantage of being more human-relevant and quicker to perform than animal studies, she said.

Christian Desaintes (DG RTD) summarised the support that the European Commission is providing for EU research programmes on alternatives to animal use and for COVID-19 research.

Maurice Whelan (JRC) talked about the challenges of bringing research communities together for cross-disciplinary endeavour and cited the recommendations of the JRC report, 'Bridging across methods in biosciences'. One initiative mentioned was the 'Integrated *in-vitro* and *in-silico* tools' (in3) project, an innovative training network funded by the EU's Marie Skłodowska-Curie action. It includes an international exchange programme bringing together young scientists to develop and promote non-animal approaches to chemical safety assessment.

The moderated discussions raised the point that animal testing was not a gold standard in comparison to non-animal methods, and that non-animal tools are increasingly considered more appropriate because they are more human-relevant.

Opening remarks

Photo: © European Commission

Virginijus Sinkevičius
European Commissioner for the Environment, Oceans and Fisheries

Q: Chris Burns (co-moderator): Polls show Europeans overwhelmingly want to reduce animal testing. How much is this conference a response to that?

A: Commissioner Sinkevičius: I think it is clear that this is a real concern to many Europeans. It's also a long-standing concern for the European Commission. In fact, and many people don't know this, it's a value enshrined in the Treaty on the Functioning of the European Union. The Treaty says that the welfare of animals must be taken into account in Union policy on areas like internal market, research and agriculture.

In addition, the Directive on the protection of animals used in science says that animals have an intrinsic value, and must be respected. Our ultimate goal is to replace the use of animals for scientific purposes, which is reflected in EU legislation.

Q: We have reduced testing, but how much could this conference and other Commission efforts, help to accelerate the 'Three Rs', Replace, Reduce and Refine the use of live animals for scientific purposes?

A: Well, we've already banned the use of animals for the safety testing of cosmetics in the EU. But there are considerable challenges in other areas.

This conference will keep the scientific debate alive, and even more importantly, it will help experts share more widely information on available alternatives. Because there is a lot to be shared on alternatives that are already available, and others that are on the way. And by showcasing progress on alternatives and engaging in debate, we hope to stimulate even more change.

The focus will be especially on looking to the future, so we are targeting the next generation of scientists. We will inform them about new developments in education on non-animal approaches, and how these can be taken further.

Q: Are there limits to the Three Rs? Certain kinds of testing that will continue, at least for now, to save human lives?

A: Of course, we all want to move faster towards replacement, but today we are not there yet. We still need to use animals for both basic and applied research, and for pharmaceutical product development, for instance. Animal research in medicine has given us many things we take for granted today, like antibiotics, anaesthetics, and organ transplants. That's true for life-saving vaccines, and it's also true for the safety testing of certain chemicals. Where there is no available alternative, we can't yet do without it, because our primary responsibility under the Treaty is protecting people's lives.

But there are developments. For COVID-19 vaccines, both animal and non-animal research is being used, showing that progress is being made. In this conference, we will see the role that non-animal methods have played in this field.

Q: Transparency is a key objective at this conference, and the theme of the first session. Can this speed up the shift to non-animal science?

A: The EU rules on protecting animals in science are opening a new era of transparency. Member States now have to share detailed reports. This means that the Commission has EU-wide statistics on animal use, and on how these animals are used, what sort of procedures they undergo.

That knowledge helps us focus research funding. It means we can concentrate on looking for alternatives in the areas where most animals are being used, and the areas where they suffer the most. We take this very seriously. The European Commission has been spending hundreds of millions of euros on research on alternatives, and we will continue to do so. The database, and the new figures, give us a good idea of where that money needs to go.

Q: Part of that transparency is to showcase examples of how researchers are reducing animal testing. How much do you think this could lead others to follow suit?

A: I very much hope that these examples – and they are only a small selection – act as an inspiration. We called the conference, in part, because we want to encourage cross-disciplinary dialogue. Often, research results stay in one discipline, like pharmaceuticals, whereas the approach could be used much more widely. We are here both to learn from each other, and to find new approaches to questions we don't yet have answers to.

Q: What got you personally involved in reducing animal testing?

A: I want to make sure we can feel good about the European approach, as this is an ethical issue. This means making sure we only use animals in science when it's absolutely necessary to save lives and protect our environment, and where alternative models are not yet available.

It also means accelerating animal replacement by investing in innovation and new approaches, also with the help of digital technologies or artificial intelligence for instance, to deliver even more reliable and ethical practices, and promote widely their uptake as soon as they become available.

Q: What would you like to see over the next two days?

A: Exchanges between scientists and decision-makers, to drive the processes further. This is crucial. Animal testing should be the last resort, so I hope that this conference will help to find alternatives and help push to the limits of using them.

Session 1

HOW TRANSPARENCY CAN ACCELERATE TRANSITION TO NON-ANIMAL SCIENCE

Co-moderator **Teri Schultz** introduced the first session, on how greater transparency can drive non-animal testing and research. The topics covered included animal statistics, public access to data, Open Science, transparency in communications, and pre-registration of research.

The speakers were: **Susanna Louhimies,** DG Environment, **Pierre Deceuninck,** JRC and **Jean-François Dechamp,** DG Research and Innovation, from the European Commission; **Serban Morosan,** League of European Research Universities; and **Zoltan Dienes,** University of Sussex. The audience contributed questions via the chat function.

Directive 2010/63/EU on the protection of animals used for scientific purposes was adopted in September 2010 (replacing Directive 86/609/EEC). The Directive is based on the Three Rs principle: Replace, Reduce and Refine the use of animals. Recently, the Commission adopted two reports under the Directive: a statistical report on the animals used, and a report on the implementation of the Directive.

Transparency on the use of animals for scientific purposes


Photo: © André Vranckx

Susanna Louhimies
(DG Environment)

Since 2010, we have a fully revised legislation in Europe that is unique in the world regarding animal use in science, said **Susanna Louhimies.** The Directive represents an important step towards achieving the final goal of full replacement of procedures on live animals for scientific and educational purposes, as soon as it is scientifically possible to do so.

This conference starts with transparency, which can help us make progress along this path, she explained. Transparency facilitates compliance and accountability, and adherence to societal values and commonly agreed rules and legislation. This builds trust between different players, and helps to combat fake news.

Importantly, believes Ms Louhimies, transparency will give us tools to provide factual data as the basis for policies and decision making: We need transparency to focus on where we need to put our efforts.

How is transparency improved through Directive 2010/63/EU? Transparency was one of three key aims of this legislation, she said, and we built new tools and obligations in the Directive to do this. These included: a) publication of operational processes and periodic information on the Member State implementation of the Directive; b) comprehensively revising the statistical reporting on animal use, making it compulsory to publish annual national data; and c) publication of Non-Technical Summaries (NTS) on projects to better inform a wider audience.

Statistics turns data into information and knowledge, which in turn can provide insight and wisdom, said Ms Louhimies. We set a baseline, identify areas of concern, and assess trends and differences between uses, severities, countries, and so forth. This is a key tool for prioritising initiatives and research efforts.

Directive 2010/63/EU was transposed into national legislation in 2013, and at this point the new, revised statistical reporting system started. In February 2020, the Commission published its first report on these animal statistics, covering the years 2015-2017[1].

The report summarises data on the number of animals used for research and testing. It also includes new information required by the Directive, such as the number of genetically altered (GA) animals created and maintained. In total, 9.58 million uses of animals in research and testing were identified and assessed by different purpose categories.

This enables us to focus on the main categories of interest for replacement and reduction, said Ms Louhimies. For refinement, for example, it shows areas where the severities of animal procedures should be reduced.

1. https://ec.europa.eu/transparency/regdoc/rep/1/2020/EN/COM-2020-15-F1-EN-MAIN-PART-1.PDF

Pierre Deceuninck, Data Scientist in the Chemical Safety and Alternative Methods Unit of the European Commission's Joint Research Centre (JRC), continued the presentation, with a more detailed look at the February 2020 report.

Pierre Deceuninck
(JRC)

He recapped the key changes in reporting obligations under Directive 2010/63/EU. The scope was extended (e.g. to include cephalopods and GA animals); each use of an animal is counted and detailed, therefore also allowing data on reuse of animals; and the actual severity experienced by the animal is recorded.

The 2020 EU report is structured into three sections to focus on species and their origins; on uses for the purposes of research, testing, routine production, and education/training and the legislative context of regulatory testing; and on the creation and maintenance of GA animals to support EU research and testing. In addition, for the purposes of transparency it contains the data submitted by the Member States.

To support regular and harmonised reporting across Member States, the Commission provides guidance in all EU languages, and organises workshops for assessing the severity of the uses of animals, explained Mr Deceuninck.

The report gives us valuable multidimensionality, he noted, and new information to improve transparency, for example, on species, severity, scientific purposes, legislative aspects, reuses, and genetic status.

In 2008, under the previous Directive (86/609/EEC), 27 EU Member States reported about 12 million animals used in research, testing, routine production, and education and training. In the new report, for 2017 about 9.4 million animals were used (first use in EU-28), a decrease of around 20%.

Of these 9.4 million animals, 61% were mice, 30% fish, 12% rats, 6.4% other mammals, and 6% birds. Non-human primates represented less than 0.3% of animals used for the first time. The report gives complete figures for all species used.

Zooming in, for example, on non-human primates, in 2017 no animals were caught from the wild, 17% were first generation purpose-bred, 53% second or higher generation purpose-bred, and 30% came from self-sustaining colonies.

The main categories for the uses of animals were 45.5% for basic research, 23% for translational and applied research, 23% for regulatory use, and about 5% for routine production, summarised Mr Deceuninck.

In terms of severity of procedures for animals, in 2017, 6% were non-recovery, 51% mild, 32% moderate, and 1% severe cases, he said; with severities higher for regulatory uses. The 2.2 million animals used for regulatory purposes were mainly used for medicinal products, both human and veterinary.

Data reveal a steady trend for the number of animals re-used in procedures, around 2%. Lastly, he concluded, of the 2.6 million animals that were genetically altered, 83% had a non-harmful phenotype and 17% a harmful phenotype.

Non-Technical project Summaries

Susanna Louhimies continued the presentation by looking at the publication of Non-Technical Summaries (NTS) of projects involving animals. NTS were introduced in Directive 2010/63/EU (Article 43) to improve transparency and help the public to understand why animals are used.

The Directive states that the NTS should be written in 'layman's language' and include: i) information on the objectives of projects, number and types of animals to be used, predicted harms to animals and predicted benefits, and ii) show a demonstration of compliance with the legal requirements to replace, reduce and refine. In some cases, Member States will update these NTS after the completion of projects to see how the results matched the predictions.

In 2017[2], the Commission reviewed the implementation of the Directive and identified several issues relating to NTS. These included animal users struggling to make them understandable to the public; authorities struggling to review and improve them; and the public having problems accessing NTS. The review concluded that the Commission should work together with Member States and stakeholders to find improved ways of making this information more accessible and searchable.

Similarly, an article from 2017 looking at ways to improve NTS, also identified issues related to timeliness, accuracy of content, accessibility, and searchability. At this time, Germany established a national database of NTS in recognition of their value.

2. https://pubmed.ncbi.nlm.nih.gov/29184966

Towards greater transparency

We can go for even greater transparency, said Ms Louhimies. We amended Directive 2010/63/EU in 2019 with a focus on further improving transparency. This was followed, in April 2020, by the adoption of a new Commission Implementing Decision (2020/569/EU) to revise the rules for statistical reporting under the Directive and establish the templates for the publication of NTS. Along with a new report on the implementation of the Directive, these will facilitate greater transparency.

The accuracy and objectivity of the content of NTS are being improved by the creation of the common template, while guidance will be provided to NTS authors. The speed of publication and access to data have also both been improved. This will be achieved by an Open Access, central EU database for NTS with the first ones due for publication in July 2021. In the coming years, this will be followed by an Open Access database for Member State level animal use statistical data (due in 2023).

However, we already have data available at EU level to help us speed up the progress toward the EU's goal of replacing animals, she said, so we have a little surprise to announce.

ALURES

Pierre Deceuninck unveiling the new EU statistical database called ALURES. Conference participants were given a 'sneak peek'.

Though we need to wait until 2023 for Member State level data to be available through the EU database, he said, the new Open Access database will soon be made available for data mining of statistics on animal use at EU level.

To start with, the ALURES database contains the same information as we have just described for the 2020 EU report, he said, but allows interactive data mining using a combinations of filters. For demonstration purposes, he focused on the database section of all uses of animals for research, testing, routine production, and education/training purposes in the EU.

Information is available through a menu on the left side of the screen, grouped into categories (e.g. species, purposes). For instance, going into a table containing information on a particular species, the right side of the screen displays all the different data dimensions, such as severity of procedures and re-use level.

Looking at Level 1 purpose of basic research, different areas of animal use can be searched, for example, the nervous system was the most common basic research use in 2017.

For regulatory uses, additional levels of information are available, for example, type of legislation and origin of regulatory requirements.

Mr Deceuninck went through some example searches. For frogs (genus Rana), in 2017, 3 500 individuals were used, for mainly moderate procedures, no animals were genetically altered, and they were almost never re-used. Example searches also used other filters, such as animal re-use and severity of procedure.

Selecting basic research, animal behaviour and biology, reveals that fish account for over half of uses (55%).

He finished the demonstration by hoping that conference participants were looking forward to using this database once it is launched.

Susanna Louhimies ended the presentation by acknowledging the hard work of her IT colleagues.

We feel that this is something that will really kick-start the work in this area, she said. Transparency has multiple benefits, she concluded. Europe has taken a quantum leap in transparency, which is one of the most powerful tools for pursuing non-animal alternatives where they are most needed.

In response to a comment in the chat, she clarified that filtering by Member States will be added to the database from 2023 onwards, in line with the revised legislation. However, she added, because we take transparency seriously, we are determined to provide access to EU-level data already now, two years ahead of schedule!


Photo: © Eva Beate Strømsted

Open Science and the European Commission

Jean-François Dechamp
(DG Research and Innovation)

Jean-François Dechamp, Policy Officer in the Open Science Unit of DG Research and Innovation (DG RTD), described how the European Commission promotes Open Science, open access and open data among researchers. His aim was to join the dots between Open Science and the replacement of animals.

The Commission wears three hats when it comes to research and innovation, he explained: policymaker, funder, and capacity builder. As a policymaker it proposes legislation and monitors its impacts; it funds Horizon 2020 (and now Horizon Europe); and it builds capacity in particular through the research activities of the JRC and the funding of research infrastructure.

Open Science means sharing data, knowledge and tools at an early stage, not only between researchers and other disciplines, but also with society at large. It can increase the quality and efficiency of research and innovation, boost creativity, and build trust in science, said Dr Dechamp.

The benefits of Open Science are wide ranging, for scientists, funders, innovative companies and society. It helps tackle the reproducibility problem; accelerates responses to societal challenges (e.g. COVID-19); yields higher impact through collaborations; and reduces inequalities. Importantly, Open Science comes with values, rights and obligations, he said.

Open Science is a pillar of the EU policy on science, stated Dr Dechamp. It reinforces the standing of our universities, research centres and innovative companies, and ensures no Member States or regions get left behind.

A growing number of EU Member States have also been putting openness at the core of their vision for research and innovation, though he noted that it requires international collaboration to fully succeed.

To highlight how Open Science is embedded in EU legislation, he referenced three key documents, all revised in 2018: i) 'Recommendations on access to and preservation of scientific information[3]', which introduced the concept of FAIR (Findable, Accessible, Interoperable, Re-usable) data; ii) 'Directive on copyright in the single market[4]', which provided an exception for research organisations to carry out text and data mining; and iii) 'Directive on open data and the re-use of public sector information[5]', which contains the reference about making publicly-funded research data "as open as possible, as closed as necessary".

From FP7 to Horizon Europe

When it comes to Open Science, the Commission started in 2008 during FP7 with a pilot on Open Access to publications. It has come a long way, with Horizon Europe soon to be officially launched. This will strengthen obligations to Open Science and responsible data management (RDM), in line with FAIR data, said Dr Dechamp.

Open Science will play a role throughout the project cycle under Horizon Europe, from proposal evaluation to final reporting. In September 2020, the Commission adopted the 'Communication on a new European Research Area for Research and Innovation[6]'. Regarding Open Science it announced the launch of a platform for peer-reviewed open access publishing (Open Research Europe[7]); and a study on authors' rights to enable sharing of publicly-funded peer-reviewed articles without restrictions; it also repeated the support to ensure a European Open Science Cloud, and to incentivise Open Science practices by improving the research assessment system.

Open Research Europe (ORE) is an Open Access publishing platform the Commission is providing free-of-charge from March 2021, to Horizon 2020 and Horizon Europe beneficiaries. It will accept publications during and after the end of grants, and provide a high-quality, reliable and efficient publishing venue, said Dr Dechamp. ORE will reduce administrative burdens for researchers, though there will be no obligation to publish there.

Articles published on ORE have to be original, stem from Horizon 2020/Horizon Europe research, and can be from any scientific area. Papers are open access with content licensed for re-use, and open peer-reviewed (i.e. reviewers are identified). By using next-generation metrics, it will be a 'super-networked' and 'mineable' platform.

The Commission set up an optional service for grant beneficiaries based on a public procurement. It signed a 4-year contract worth €5.8 million in March 2020 with a consortium led by the service publisher F1000 Research[8], explained Dr Dechamp. F1000 Research will provide the open research publishing infrastructure, and OpenAIRE[9] will act as a partner to help with syndication of articles in ORE.

3. https://ec.europa.eu/digital-single-market/en/news/recommendation-access-and-preservation-scientific-information
4. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2019.130.01.0092.01.ENG
5. http://data.consilium.europa.eu/doc/document/PE-28-2019-INIT/en/pdf
6. https://euraxess.ec.europa.eu/euraxess/news/new-era-research-and-innovation
7. https://open-research-europe.ec.europa.eu/
8. https://f1000research.com/
9. https://www.openaire.eu/

Communicating Animal Research at Universities


Photo: © Serban Morosan

Serban Morosan
(LERU/Sorbonne University, France)

Serban Morosan summarised the Note from the League of European Research Universities (LERU) on 'Good Practice in Communicating Animal Research at Universities'[10]. At LERU, we think it is very important that scientists explain to the public how animals are used for science, and about non-animal methods in science.

Founded in 2002, LERU is a network of 23 research universities based in 12 countries around Europe. The Note published in December 2020 sends a strong message to universities that open and transparent approaches to animal research can help increase the awareness of the public about animal research, he said.

There have only been a few specific surveys on public perceptions to animal science, explained Dr Morosan. However, these indicate the need for more public education about animal research.

A step in the right direction is provided by the Concordat on Openness, which was launched by Understanding Animal Research in May 2014. In 2020, 121 organisations had signed its agreement on transparency and openness. In my opinion this was the starting point in Europe for transparency agreements, he said.

Today, in collaboration with EARA (European Animal Research Association), 179 organisations have signed transparency agreements on animal research, in Spain (2016), Portugal (2018) and Belgium (2019). This will soon be extended to France (2021) through a transparency agreement launched by GIRCOR, a French national animal research advocacy group. Discussions are ongoing in Switzerland, Italy, Poland and the Netherlands.

Transparency agreements typically have four commitments: i) to be clear about how, when and why the biomedical sector uses animals in research; ii) to enhance communication with the media and the public about research using animals; iii) to be proactive in providing opportunities for the public to learn about animal use and the regulations governing it; and iv) to report on progress and share experiences.

Previous reference documents about communicating animal research have been published by Understanding Animal Research, EARA and others. An EARA study of EU-based websites in 2020 concluded that institutional websites were a good tool for informing the public, media, decision-makers, and regulators about the use of animals in research.

Dr Morosan noted that public opinion appears divided on the use of animals in all types of research, even where there are no alternatives. He said that many universities are still hesitant towards openness and transparency, while others are now experiencing the advantages that openness and transparency can bring.

For LERU, transparency is essential for developing trust, and it is the basis for public support for continuing work with animals until they can be replaced by alternatives. Universities could be more vocal about the benefits animal use brings to basic and applied research, said Dr Morosan.

In its Note, LERU outlined several actions that universities can take to improve transparency and communication: i) establish an active Animal Research Communication Group; ii) develop an efficient communication plan and strategy; iii) outline key messages; iv) provide accessible section on animal research on university website; v) hold public outreach events; and vi) develop a strategy to act on campaigns/protests against animal experimentation.

10. https://www.leru.org/publications/good-practice-in-communicating-animal-research-at-universities

Pre-Registration of Research

Zoltan Dienes
(University of Sussex, UK)

Zoltan Dienes, Professor of Psychology at the University of Sussex, talked about an area of publishing of growing importance. It involves researchers sending reports for approval to journals that set out what they plan to do, before they do it.

In 2013, the Cortex journal set up a pioneering registered report site, which strictly enforced the principles of pre-registration. Today, 277 journals have registered reports as an article type.

Authors send manuscripts to editors of these journals without yet collecting data, explained Prof Dienes. They send the introduction, methods and the planned analytic protocol. This is then nailed down on acceptance.

For Cortex (a journal devoted to human cognition studies), for example, after editorial approval, the reports are sent to reviewers who comment on the proposed methods, before the study is run. So you have the benefit of authors, editor and reviewers collaborating, to make it the best experiment it can be, he said.

Once it has been accepted at Stage 1, called In Principle Acceptance (IPA), authors are free to collect the data, but only using the accepted method and analysis. Once the data has been collected and analysed, authors submit Stage 2 manuscripts. Reviewers confirm (or not) that the Stage 1 protocols were followed and if the conclusions reached are justified. The authors can do additional analyses, if it is transparent and clear, but this must go in a separate section of the paper.

What this means is that the paper is accepted for publication no matter what the results are, said Prof Dienes, and this avoids the bias in the publication record toward positive results. This helps science, and it helps authors who get a guaranteed publication independent of the results.

One way it is good for science is that it prevents researchers rewriting the narrative in the light of the data. Authors can no longer, after data collection, rewrite earlier sections of their papers to push the statistical significance of the data towards the outcome they would like to have, to make positive results more likely (P-hacking).

A key pressure on authors is the need to publish in high-impact journals. Registered reports have overcome this problem because predictions and the analytic process is set in advance. In the context of animal research, all the data is published transparently, is genuinely valuable and reproducible.

Prof Dienes summarised results from two papers published in 2019 that compared predictions from the standard psychology literature and registered reports. Predictions were achieved in 96% and 80-95% of cases in the standard literature, but, more realistically, in 44% and 40% of cases for registered reports.

PCI (Peer Community In) offers another model, he said. It is a free recommendation process for scientific pre-prints based on peer-reviews. PCI Registered Reports launches on 19 April 2021. Editors, here called recommenders, send pre-prints to reviewers. Submissions are edited until they can be recommended for publication in one of 12 PCI-friendly journals. These journals are committed to accepting them, as long as the pre-stated criteria are met.

Moderated discussion

Teri Schultz *(co-moderator) started the discussion by asking for more information about searching for Member State information on the database, and the languages used.*

Susanna Louhimies *(DG Environment) stressed that it is important to distinguish between the statistical database and the Non-Technical Summaries (NTS) database. On the former, reports for different Members States can be obtained, based on equivalent data collection methods. Data from before 2015 is not included, because of its poorer quality. Therefore, when data mining is opened to the public, we can feel confident with the quality of the data. We still go back to Member States when potential errors are detected. It is a continuous work in progress, she said. For the NTS, users write them in their own national language, and these are submitted to the database. The Commission will provide a translation tool in the background to accommodate key search words in all official EU languages.*

Celean Camp *(Frame): Presumably data from the UK has been included in the database to date. Will it continue to be included post-Brexit? If not how will we be able to produce accurate trend analyses?*

Susanna Louhimies: *The UK data will be provided from 2015 until, and including, 2019. Concerning trends, Norway has also implemented the Directive and from 2018 data onwards, its data will be included in the database. When comparing trends, such factors need to be taken into account. For example, Norway is an important user of fish, which will introduce significant annual variations in the statistics.*

Teri Schultz: *Is data coming from private companies inside the numbers published by Member States?*

Susanna Louhimies: *Yes, though there is one exception, which is animals used for the purposes of national defence. These numbers are not covered by the EU statistics, as national defence is an exclusive competence of Member States.*

Chris Burns *(co-moderator), channelling comments from the chat, asked what academia can do to ensure industry is more transparent about animals?*

Serban Morosan *(LERU): Universities can only send a strong message, a lot of collaboration with private companies and to share information and have good agreement will be a very good start. In the agreement about transparency, the 120 organisations include academic and private ones.*

Valeska Stephan *(Commission on Animal Protection and Experimentation, German Research Foundation): What is meant by 'protection' in relation to Open Science?*

Jean-François Dechamp *(DG RTD): Open Science does not mean everything that goes open is unprotected. You have to protect your rights. A creative commons licence, for instance, is a way of marking your results to receive credit[11].*

Aleksandra Badura *(Erasmus MC): Is there data on who reads the NTS? For citizens without scientific background, the information is still hard to digest. Could a more user-friendly format be used, like videos?*

Susanna Louhimies: *It will be interesting to see who accesses the database. I hope we will be able to start collecting visitor and search information. We hope the NTS inform the public, but are also helpful to NGOs and researchers.*

Gilbert Schönfelder *(German Centre for the Protection of Laboratory Animals (Bf3R)) noted that YouTube video guides on NTS already exist in German[12].*

Serban Morosan: *In the website survey, it was found that different ways of communicating, like videos and case studies, helped explain in layperson language.*

Miriam Zemanova *(Animal Free Research): There are issues with inconsistent formatting and unavailability of NTS from some of EU Member States. What will be done differently in the future to enforce transparency?*

Susanna Louhimies: *EU law is transposed into national legislation. Therefore, it is the competence of Member States to enforce their national legislation. However, if we see systematic non-compliance, the Commission has the tools to intervene. Another comment concerned the time taken to publish NTS. It is important to know that before the 2019 amendment of the Directive there was no deadline for publication. We noted this as a problem, so now the Directive requires the publication within six months from the authorisation of the project.*

Teri Schultz *relayed a question asking if peer-review causing papers with negative results to be rejected is still a concern in the new publishing model.*

Serban Morosan: *For me, a negative result is a result. If we share negative results for a start within LERU's 23 universities we have a lot of important data. I think negative results increase transparency and can help drive the transition to non-animal science.*

Zoltan Dienes *(University of Sussex, UK): Regarding negative results, a registered report is published regardless of its results. There is far more transparency than exists with other article types, all through the system. Some papers submitted are rejected at some point; but mostly these studies were not run, they were just proposals.*

Susanna Louhimies: *I really applaud this idea of registered reports. I think it is something the animal user community should embrace, to ensure every study is published. The question of being rejected should only be down to scientific robustness, not depending on whether the results were negative or positive – as both results increase our knowledge.*

Chris Burns *asked what the quality guidelines are for registered reports.*

Zoltan Dienes: *Most of the journals have similar guidelines, based on what was originally written by Chris Chambers for Cortex. If you go to Peer Community In registered reports[13], you will find very detailed guidelines for this new platform, including things like manipulation checks and outcome neutral tests.*

Susanna Louhimies: *I think these are important things to implement in one's own*

11. The Commission adopted Creative Commons under its re-use policy in February 2019: https://ec.europa.eu/transparency/regdoc/rep/3/2019/EN/C-2019-1655-F1-EN-MAIN-PART-1.PDF
12. https://www.youtube.com/channel/UCMVclcqKizavpBYywHbhrIA/featured
13. https://peercommunityin.org/

work, regardless of where the results are published. In addition, the ARRIVE guidelines have just been revised, and they provide an important tool to improve the reporting of in-vivo studies. People planning animal research should also look at Norecopa's PREPARE guidelines[14].

***Teri Schultz** asked if more transparency necessarily equals less animal use.*

***Zoltan Dienes:** There are many studies unpublished because the results did not come out positive or as expected. That is a clear waste of data. Someone not knowing about it, may try to redo it. Registered reports help avoid this duplication, because everything with a sound methodology gets published.*
Jean-François Dechamp: Transparency is the key. We have also been working on the issue of re-usability of data. By promoting the openness of data, it enables the data to be really re-used.

***Chris Burns:** Should ethics committee minutes/inspection reports and follow-ups be published in the spirit of transparency?*

***Susanna Louhimies:** This was discussed at length when the Directive was negotiated. The consensus was that the reports contain so much detailed and technical data that it would not be helpful for the general public.*

***Bogdan Sevastre** (University of Agricultural Science and Veterinary Medicine Cluj Napoca): The documents of ethics committees might also contain confidential data not suitable for the general public.*

***Penny Hawkins** (RSPCA): Ethics committees should also be a 'safe space' where people can openly discuss any concerns; redacted summaries of meetings facilitate this.*

***Chris Burns:** The cosmetics sector has stopped animal testing. Which sectors are advancing quickest to be next?*

***Susanna Louhimies:** We can look to areas where the most efforts are put in, and what science is allowing us to do. In the past, we have had success in the area of topical toxicity, which have allowed for transfer into non-animal methods. Other sectors have more complicated approaches. However, where alternatives exist, EU legislation requires their use.*

***Gilbert Schönfelder:** Beyond registered reports, we also need animal study registries. Can you comment or say that we see their value as well?*

***Susanna Louhimies:** Some recent initiatives are emerging. We have the Animal Study Registry in Germany, and another initiative on pre-clinical trials in the Netherlands where animal study reports have been registered.*

***Nicolas Guy** (CNRS): What are the limits (if any) to transparency in this field?*

***Susanna Louhimies:** There are limits to transparency. We have to ensure there is proper protection of intellectual property rights; and there are issues in terms of extreme activism, to protect names and addresses of scientists. It is also important to protect competitiveness, academic or private interests, and keep some details confidential, but ensure information can otherwise be utilised. We have to find a balance.*

***Zoltan Dienes:** For the example of brain imaging, there is also the issue of not identifying individual patients.*

***Teri Schultz** asked about lab inspection reports. Should they be covered by transparency?*

***Susanna Louhimies:** We do have some information on inspections at EU level through Member State implementation reports submitted to the Commission once every 5 years. The first EU report was published in February 2020 containing a section on inspections. It includes important information such as numbers of inspections, proportion of unannounced inspections, as well as summaries of main problems detected during inspections.*

Final thoughts

***Jean-François Dechamp** (DG RTD): I can say that open access has been a game changer in the world of scientific communication. Thirty years ago, there were only subscription journals. The Commission wants, as a funder and with its other roles, to ensure venues of quality for open access publications are available to academics. ORE is a high quality, free-to-use, fully open access platform, with open peer review. I really hope researchers embrace all the different possibilities offered to publish their results, and move away from journal impact factor considerations.*

***Serban Morosan** (LERU): The next step will be to harmonise all these types of communication. But, to be clear, in terms of transparency and openness, we have to talk about both animal research models and non-animal alternatives. It is important that the Commission helps with initiatives aiming to publish negative results. The next step is to harmonise local and national university initiatives to share negative results between 23 universities. If we can eventually harmonise this among all European players it would be great.*

***Pierre Deceuninck** (JRC): I would like to stress that sharing data is extremely important for transparency. There were lots of questions on how to use this data, but this data is going to be public. As soon as data leaves the EU databases it will start to be used and put into new contexts. It is work that is becoming increasingly important, and I think we have seen the hard work being done to build such a database. I think it is a tool that will play an important role in the coming years for replacing animals in science.*

***Zoltan Dienes** (University of Sussex, UK): Just to add something on incentivisation. One crucial aspect is the funder, who creates opportunities and requirements for open access and open data, but could also make arrangements with journals so that the process of getting a Stage 1 acceptance is also the process by which that study is accepted with regard to receiving a grant. This would link registered reports to the funding process.*

***Susanna Louhimies** (DG Environment): We talked about expanding transparency from local to global level. I think that this should really be our ambition and we hope that Europe has been pioneering in this field. We are going to encourage other regions to follow these steps - because research and testing are not local, but global activities. We need to work together and I am really looking forward to the initiatives and actions by all involved as we start making use of this data.*

5 discussion points

- ***Open access has been a game changer in scientific communication.***
- ***Registered reports ensure every study is published independent of results.***
- ***A negative result is a result. Negative results increase transparency.***
- ***Could registered reports also be linked to the funding process?***
- ***There are limits to transparency, such as protecting competitiveness and intellectual property rights.***

14. https://norecopa.no/PREPARE

Session 2

EDUCATION & TRAINING WITH A FOCUS ON NON-ANIMAL APPROACHES AND IMPLEMENTATION OF THE DIRECTIVE

This session focused on the importance of education and training in delivering the Three Rs. The topics were: training aimed at schoolchildren, university students and researchers; and alternative methods to using live animals for teaching and training, such as plastinated models and virtual reality.

The speakers were: **Katrin Schutte,** DG Environment, European Commission; **Daniela Salvatori,** Head of Anatomy & Physiology Veterinary Faculty, Utrecht University; **Julia Malinowska,** University of Birmingham; **Marcelle Holloway,** Joint Research Centre, European Commission; and **Lindsay Marshall,** Humane Society International.

Katrin Schutte
(DG Environment)

Open access training E-modules and other education resources available via ETPLAS

Katrin Schutte talked about recent education and training tools developed on the Three Rs and Non-Animal Alternatives (NAMs). In particular, she introduced the Education & Training Platform for Laboratory Animal Science (ETPLAS).

Education and training is a legal requirement under Directive 2010/63/EU, as competence of staff is central to good animal welfare, she said. Before the Directive, there was no harmonised EU training and education in laboratory animal science; though isolated courses existed in Member States. The Directive now provides a framework that all Member States can use.

The EU Education and Training (E&T) Framework (2012) creates a common understanding and a needs-driven training and assessment framework. It has a flexible, module-based structure, making it easily accessible and affordable, and its content is of an agreed quality. It will ensure the competence of staff, and facilitate free movement of researchers, animal technicians and care givers, said Dr Schutte.

With funds received from a European Parliament pilot project on education and training for the purpose of promoting non-animal alternatives, we have contracted the development of six interactive E-learning modules, as well as a contract to support the development of the Education & Training Platform for Laboratory Animal Science, ETPLAS, so that this platform can become the hub of training information in Europe, said Dr Schutte.

Of the six E-modules, the first four focus on the implementation of Three R elements (EU-10, EU-11, EU-12 and EU-25). The other two E-modules are new and concern non-animal alternatives (EU-52 and EU-60). The first four have been available on the ETPLAS website (etplas.eu/learn/) since January 2021; the other two will be available later in 2021. The modules make clear reference to how their content fits with the E&T Framework that serves to fulfil the requirements in Articles 23 and 24 of Directive 2010/63/EU.

The E-modules are structured to be suitable for both self-learning and incorporating into a training course; provide information in a way that facilitates learning (e.g. multimedia, interactive exercise and knowledge checks); and encourage further learning through easily accessible references.

'EU-10: Design of procedures and projects – level 1' introduces students to key aspects of experimental design, such as variability, sample size, statistical methods, different study designs, and the importance of randomisation.

'EU-11: Design of procedures and projects – level 2' goes into more detail concerning the planning of projects, like why use animal models, how to apply human endpoints, and prioritising different Three Rs when they come into conflict.

'EU-12: The severity assessment framework' provides information on the prospective classification of the severity of procedures involving animals, the continuous assessment of severity, and the reporting of actual severity.

'EU-25: Project evaluation' provides guidance on aspects that competent authorities need to consider, such as principles and criteria of project evaluation, ethical and welfare issues, and how to formulate well-informed, impartial and justified opinions.

EU-52 is a new module being developed on searching for non-animal alternatives. It will enable students to distinguish different types of replacements, and explains how to design and implement a search strategy.

EU-60 will focus on developing non-animal alternatives for regulatory use, from start to finish. It will cover *in-vitro* methods, how to apply OECD Good *in-vitro* Method Practices (GIVIMP), and how to demonstrate the validity of a new method.

In addition to hosting the E-modules, ETPLAS is being developed as a unique one-stop shop for education and training in laboratory animal science in the EU. In 2016, ETPLAS received a share of the funding from a European Parliament pilot project on education and training for the purpose of promoting NAMs. This was used, for instance, to develop a library of Direct Observation of Procedural Skills (DOPS) for assessing competence, and for building sustainability to make all the tools being developed available to a diverse user community.

The first four ETPLAS E-learning modules (EU-10, EU-11, EU-12 and EU-25 were shown during the lunch breaks of the conference. The modules were developed in collaboration with FLAIRE Consultants, Scientialis, PNBN Enterprises, and Envelope Design. These E-modules were introduced by Paul Flecknell of Newcastle University (UK) and Director of FLAIRE.

Anatomical models for Education and Training: focus on plastination and virtual reality

Daniela Salvatori
(Utrecht University)

Daniela Salvatori, Head of the Anatomy and Physiology Department at the Veterinary Faculty of Utrecht University, the Netherlands, talked about how plastinated models and Virtual Reality (VR) are replacing the use of live animals in education and training in the veterinary curriculum. These methods could be extended to other universities and courses.

Traditionally in the necropsy and anatomical room, we have used cadavers, she said. Beside ethical concerns, there are problems relating to the large storage space required and the use of the preservative formalin that is dangerous for humans. In the Netherlands, we use more than 15 000 animals every year for training and education. Our students need to know about eight species in great detail. There arose the question of whether there were better methods for them to learn?

Therefore, we created the Centre of Excellence for Plastination and Virtual Reality at Utrecht University, explained Prof Salvatori, with the aim of rapidly exchanging knowledge on these techniques among faculties and students.

Proefdiervrij (Dutch Society for the Replacement of Animal Testing) started the project Dierdonorcodicil (Animal Donor Codicil) in collaboration with the Centre of Excellence. This enables pet owners to donate the bodies of their deceased pets to the facility, she said. These cadavers are used to make hard and soft plastinates, using techniques that replace body fluids with polymers such as silicone rubber.

Plastinates are long-lasting and can be used for a wide range of teaching purposes. Hard plastinates are useful when teaching anatomy, while soft plastinates are ideal flexible training models for clinical procedures such as giving injections. We have introduced a lot of these models within our education programme, she said.

At the same time, we are working on Virtual Reality models, using Microsoft HoloLens and Aryzon technology, said Prof Salvatori. Wearing VR headsets, students can effectively move around a 3D projection of an animal. The first model to be developed was a rat. By customising the headsets, students are provided with information alongside the avatar animal, in an interactive side bar that gives them all the required information on anatomy, physiology, histology and pathology.

We are working on VR models that also have physiological movement, she said. Groups of students can interact with the virtual models, and by inserting mobile phones in the headsets, information can be downloaded for further study.

Plastination and Virtual Reality are changing teaching practices. In the Faculty of Veterinary Medicine, there is now a complete curriculum at Bachelor's level that can be taken without the use of live animals for teaching and training, she said. Using these approaches has reduced the total number of animals used, by at least 60% in teaching and training, especially for anatomy, concluded Prof Salvatori.

Learnings from JRC Summer School on Alternatives


Photo: © David Epps

Julia Malinowska
(University of Birmingham)

Julia Malinowska introduced herself as a final year PhD student at the University of Birmingham (UK) in the School of Biosciences, working on the development of high-throughput *in-vitro* metabolomics approaches to aid animal-free chemical safety assessment, in the research group of Prof Mark Viant.

She talked about her experience of attending the JRC Summer School on 'Non-Animal Approaches in Science – Challenges and Future Directions', held in May 2019 in Ispra on Lake Maggiore (Italy).

Among the reasons she applied for the Summer School were to learn more about non-animal approaches in toxicology and biomedical science; to connect with others working in this area; to promote her own research and the use of 'omics in non-animal studies; and to investigate the job market in the area of the Three Rs.

Her initial impression was of positive surprise at the diversity of participants, both in terms of their countries and the work they do. She described the Summer School as an intense and rewarding four days. It comprised talks, workshops, lab tours, debates, and poster sessions where participants presented their own research.

Ms Malinowska also described attending another Summer School that was online, on 'Innovative Science without Animals', jointly hosted by Johns Hopkins University (USA) and the JRC in 2020. Online Summer Schools improve accessibility, she said, but networking is best in person. She noted the potential for future hybrid courses.

She summarised the many benefits of attending the Summer School in Ispra in 2019. These included promoting your own research; connecting with experts; networking and exploring collaborations; and understanding wider job opportunities relating to the Three Rs; not just academia and industry, but also regulatory and legislative work, education and training, outreach and science communication.

From her experience, Ms Malinowska highlighted the importance of the JRC Summer School in furthering the aims of accelerating progress in the Three Rs, as set out in their Science For Policy Report[15] .

I incorporate the learnings in my research and other parts of my life, she said. First, increased knowledge makes me more confident when I talk about the Three Rs, and how I champion them locally, for example, in talks and posters, and when supervising undergraduate students. It helped me crystallise my career path related to the Three Rs, as well as helping me narrow down the projects that I undertook as part of my PhD in order to make it more Three Rs relevant.

I would highly recommend the JRC Summer Schools. It was one of the highlights of my entire PhD. For me, it was a game changer, concluded Ms Malinowska.

15. https://publications.jrc.ec.europa.eu/repository/bitstream/JRC103906/jrc103906_3rs_ks_science_for_policy_report_final_online.pdf

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


Photo: © Marcelle Holloway

Marcelle Holloway
(JRC)

Marcelle Holloway talked about how the JRC is promoting the Three R concept in education programmes. The JRC has its headquarters in Brussels and research centres in five EU Member States: Belgium (Geel), Italy (Ispra), Germany (Karlsruhe), the Netherlands (Petten) and Spain (Seville). The EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) is in Ispra.

ECVAM works on research into and validation of alternatives to animal testing, but also on dissemination and promotion to support the mandate of Directive 2010/63/EU, she said. This includes initiatives like the JRC Summer Schools and other training activities. One of the breakout groups at the conference featured an escape room-style lesson from their upcoming Summer School in May 2021 (see Breakout Group 3, p. 28).

Thanks to the support of the European Parliament pilot project and DG Environment, we have been able to step up our education work in the Three Rs, she noted. This includes education initiatives for better integrating the Three Rs in the classroom, targeting secondary schools, university students and the training of early career professionals.

We found that education and training is key to good Three R knowledge sharing, said Dr Holloway. If you successfully share work and knowledge, you boost the application of the Three Rs. This in turn improves animal welfare, reduces animal use and eventually replaces animals - the ultimate goal of the EU.

A mapping exercise that started in 2018 revealed an abundance of teaching resources on the Three Rs and alternative methods. However, they were unevenly distributed, with very little provision for secondary schools, she said. The JRC have been looking to actively integrate Three Rs into education programmes, with a special focus on secondary schools and with supported actions at higher education levels.

The JRC Report 'Introducing the Three Rs into secondary schools, universities and continuing education programmes'[16] was published this morning, said Dr Holloway. The report, produced in collaboration with 15 education experts and Three Rs experts, highlights the importance of teaching the Three Rs, and how to further integrate this aim in teaching.

The report looks at ethical aspects of animal use, she said, and how innovative non-animal science developments are evolving fast and opening up exciting new career paths and job opportunities for young people. It also covers the legal framework in the EU, and how the Three Rs are relevant to several disciplines beyond science, for example, history, politics, law, economics, and business studies.

We make recommendations about how you can integrate the Three Rs better using, for example, outreach activities, she explained. The report is aimed at decision-makers and education influencers, who stimulate and facilitate the development and uptake of new educational content and resources.

The strategy outlined in the report relies on the provision of resources. To this end, the JRC developed a MOOC (Massive Open Online Course), 'The Three Rs and Animal Use in Science'[17], aimed at life science teachers in secondary schools, though it is open to everyone. This was completed early in 2020, in collaboration with European Schoolnet and external partners.

The MOOC is based on six learning scenarios, developed with teachers in pilot exercises, explained Dr Holloway. These aim to develop critical thinking skills, Three Rs and alternative technology knowledge, and science literary skills. Learning scenarios are presented in a way that structures the information to help teachers prepare lessons on any subject.

The real-time MOOC includes live sessions and webinars, and it will be repeated during 2021. However, the static content can be viewed at any time.

We have had 264 teachers participating, she said, and we estimate that we reached out to 8 000 students in 2020 alone. European Schoolnet gave a live lesson based on one of the learning scenarios from the MOOC in one of the conference breakout sessions (see Breakout Group 1, p. 26).

For me this is genuine outreach, reaching out to a community that does not know anything about this subject. They are taking it onboard, running with it, and teaching it, said Dr Holloway.

There are another six learning scenarios in place which can be used by universities and professionals, including module-based ones for skills training, she said. The JRC Summer School was even transferred into a learning scenario, so others can build their own summer schools. All learning scenarios are in the JRC data catalogue[18].

16. https://publications.jrc.ec.europa.eu/repository/bitstream/JRC123343/jrc123343online_1.pdf
17. https://www.europeanschoolnetacademy.eu/courses/course-v1:3Rs+AnimalsInScience+2020/about
18. https://data.jrc.ec.europa.eu/dataset/5803050b-bdc4-4032-bbda-f794a0fc58c0

Learning to apply non-animal, new approach methodologies in human health research

Lindsay Marshall
(Humane Society International)

Lindsay Marshall talked about a new initiative by Humane Society International on new approach methodologies (NAMs) in human health research. It is being conducted with BioMed21 and involves external collaborators, including Kathrin Hermann of Johns Hopkins University (USA) and Brett Lidbury of The Australia National University.

They are developing an educational course that targets a broad audience, with the aim of improving the visibility of, and confidence in, NAMs. The focus is on the Replacement of animals in research, though they acknowledge the excellent efforts for Reduce and Refine that are steps on this pathway, she said.

We are looking at how NAMs are being used to address different research questions in human health, explained Dr Marshall, who is the Biomedical Science Advisor of the initiative. The online course will enable researchers to look at the range of tools available, with respect to their own research questions, and it will provide easy-to-understand information for anyone wanting to find out more about NAMs. The independent course, not associated with any particular institution or university, will be created as an online platform. We are trying to make it as engaging, interactive and attractive as possible, she said. To this end, it will have a flexible structure that can be accessed at any point.

Those engaging with the course will: i) be made aware of the range of non-animal based tools available to address specific research questions; ii) understand how these tools offer a more human-relevant and human-predictive approach; and iii) get an understanding of the landscape of new approach methodologies in routine use, where methods still require more development, and where there are gaps that require novel method creation.

Dr Marshall described the course layout, based on a series of questions to direct the visitor to relevant information, and subject topics that link to short explanatory videos. On the boxes for each NAM, drop-down menus list all the resources associated with that method. She showed an example video, on microdosing.

The online course will also link to additional reading, such as papers and books, and other resources, like webinars, suppliers (e.g. of cells and serum), and databases like EURL ECVAM's Database on Alternative Methods to Animal Experimentation.

This is a work in progress and we want it to be useful, concluded Dr Marshall. Please let us know what you think of it. All feedback will be gratefully received: What have we missed out? Would you like to help develop this further? Would you like to host/use/adapt the course when it is ready? An online questionnaire is available to supply feedback[19].

19. https://docs.google.com/forms/d/e/1FAIpQLSfKSN8NQXfWUHlc10-FNLk3AYK_cbgmypwvTfqip-JsADsv57g/viewform?vc=0&c=0&w=1&flr=0

Moderated discussion

***Teri Schultz** (co-moderator): How can the Commission monitor Member State compliance with the Directive's requirements? Article 23(3) requires Member States to publish minimum requirements for education and training for people dealing with live animals in science.*

***Katrin Schutte:** The Commission is checking everything that Member States transpose into their national legislation as part of the conformity checks. Member States were expected to publish training requirements when Directive 2010/63/EU came into force, so any such incompliance would be picked up in a conformity check.*

***Marianna Rosso** (University of Bern): At what level of education should these modules be implemented? The earlier the better?*

***Daniela Salvatori** (Utrecht University): I am not sure if I can give an age. We have a plan for education that covers academic and non-academic education, and flexible programmes that can be used at different levels. At Utrecht University, we have the ambition to work with the schools that train our laboratory animal technicians, our care takers, and to have a programme that can link to university, so of course we can start very earlier, but I also think the academic and non-academic world can link better together and have a very solid programme.*

***Teri Schultz** asked Julia Malinowska at what point she became acquainted with non-animal methods. At 14, I remember being asked to dissect a frog and a baby pig in high school, I would much rather have had plastinated models. How young is too young?*

***Julia Malinowska** (University of Birmingham): I think it is difficult to put an exact age, but I do think it should go pre-university to make students aware of non-animal research and that there are other ways of doing research. I became familiar with non-animal research when I started my PhD, but I wish I became aware of it sooner.*

***Marcelle Holloway** (JRC): The JRC MOOC was aimed at Life Sciences students 15-18 years, to give learning scenarios in classes, but it was open to everybody. We had other stakeholders present in the MOOC and it was also of value to them. It is the way it is presented and marketed.*

***Teri Schultz:** Does the Commission have plans to support further programmes on e-learning?*

***Katrin Schutte:** We are only trying to be a facilitator in this process of offering training, it is not a principle area of activity. However, we are happy to connect players, and to make sure resources can be pooled and as many connections as possible can be formed. In the coming years, beyond the modules mentioned in my presentation, we have a plan to produce more modules on specific skills and techniques, and other aspects within the training framework.*

***David Beehan:** Do trainees completing ET-PLAS training modules get a certificate of completion?*

***Katrin Schutte:** That is not in place yet, but we have started to discuss this.*

***Q:** How long do you estimate it takes to get through the Humane Society International course? How much biology background is required?*

***Lindsay Marshall** (Humane Society International): There is no linear route through the course, so the time taken depends on how much you want to get from it. You could enter for 10 minutes and get the information you need, or take time to explore in depth. Anyone with an interest would understand the introductory videos, for example, but the case studies are really for people designing their research without animals. As for whether you can be too young, I would be careful not to scare children with something they had not considered before. How you pitch the message is important.*

***Q:** What other applications could you see for virtual reality tools in education and training to advance non-animal approaches?*

***Daniela Salvatori:** This technology offers a wide range of possibilities. For example, we are building an avatar dog to show the anatomy of the locomotor system. Virtual Reality can have numerous applications in pre-training for procedures before moving to animals. There is a need to develop research on the efficacy on holographic teaching models, which are easily shareable once put on a platform.*

***Teri Schultz:** How do you simulate an operation or disease?*

***Daniela Salvatori:** With plastination we can have normal and pathological models for comparison. Virtual Reality can be overlaid on an operation. Imagine a surgeon who needs to find a special nerve or make a ligation of a vessel, the idea is that the surgeon works the HoloLens and the headset can look for these details. In the Netherlands, a teaching hospital has introduced virtual reality in their medical curriculum, for example, a VR app to recognise when the heart or lungs are functioning incorrectly. In the veterinary curriculum, we are a bit behind this.*

***Tomasz Sobanski** (European Chemicals Agency): Proper training in alternative methods (especially for systemic toxicity endpoints) requires quite an extensive and multidisciplinary skillset covering molecular biology, analytical chemistry, toxicology, data processing and modelling, and statistics. Do you have any experience or idea how to pass this knowledge to the students in an efficient manner, without overwhelming them?*

***Katrin Schutte:** I don't have practical experience, but I have an idea of how it could be started. The teaching would need to start with the understanding that we have all gained since we started working on alternatives. What we understand better is the cause of an effect. What molecular mechanism or physiological mechanism leads to an effect causing harm in an organism or causing positive effects of medication in an organism? Given that we understand these better at a molecular level, we could start tailoring that information to, for example, explain to a school student what happens at cellular level before you develop an allergy, then show schematics at what happens in cells in blood rather than telling them the ugly skin reaction you see in this photo is an allergy. So, it is a completely different approach to how you explain science. I think this change is already happening in schools.*

***Chris Burns** (co-moderator): When you make your MOOCs how do you do it without overwhelming people?*

Marcelle Holloway: *I think an important thing to mention about the MOOC we have done is that we wanted teachers to co-create them, because they know what works and does not work to prevent students being overwhelmed with information.*

Kathrin Herrmann *(Johns Hopkins Center for Alternatives to Animal Testing): I am a veterinarian and of course you eventually need to work on animals. But you do that as one does as a physician. You learn on the side of an expert. Under supervision.*

Teri Schultz: *All the mistakes you make, the practicing could be done on plastic, but at some point you need to work with live animals to have sufficient training?*

Daniela Salvatori: *Yes, we have to be realistic about facing this topic. We at Utrecht have replaced all animals at the Bachelor's level.*

At the Master's level, we strictly reviewed the learning goals of each single course using animals and also there we have tried to make choices according to the learning goals. Of course, a vet needs to cure live animals so at some point we need to use live animals. But, during the course and training we can drastically decrease the number of animals used. Marcelle Holloway: I agree. I think at the moment it is a question of reduction, using the alternatives, like plastinated animals, as far down the line as you can.

Julia Malinowska: *I could not agree more with the previous comments. I think a lot of that is based on expert judgement. It is perfectly reasonable to just use plastic models during internships, then start working on live animals as late as possible. This highlights the importance of non-animal research as well.*

Lindsay Marshall: *You won't be surprised to hear that I disagree slightly, though I have to qualify that with saying I have not been involved in vet training. From my background of biomedical research, I would like to see us moving to a place of no animal use at all, because for me the species in question is human and we need to get better human-predictive models and we will not get them with animals.*

Katrin Schutte: *It really depends on the type of skills you are training for. If you are training to be a vet then I support Daniela Salvatori, but I would agree with Lindsay Marshall that we can be more creative in reducing and refining further.*

Breakout Group 1

High School: Three Rs in schools in Europe


Photo: © European Schoolnet


Photo: © Katarina Jokićević

Agueda Gras-Velazquez and **Eleni Myrtsioti** from European Schoolnet (EUN) reported back on the demonstration of the 'The Three Rs and Animal Use in Science' MOOC.

During this breakout session, European Schoolnet with the support of two high school teachers presented the Three Rs Learning Scenarios and implemented one live with the participants taking the role of students. The 'students' broke up into four teams, where they worked on how would four different persona defend their views on using Animals in research: a) Patient with untreatable disease; b) Pharma company CEO; c) Animal lab scientist; and d) Animal rights activist, which they then shared with the whole group.

Dr Gras-Velazquez summarised the main interesting outcomes:

- It is important to remember that teachers are not experts in the topics they teach, so the collaboration with experts to review the content / scientific part is always needed.
- At the same time, teachers are the experts in education and managing classes so they know better how to adapt the topic, what can be explained and how in class, and how to animate the lessons.
- In general, but especially when talking with students and debating a topic in class, with the aim to develop critical thinking, you need to provide an environment that allows for different opinions backed up by science/research to be expressed without judgement or censorship to ensure everybody feels comfortable to disagree.

As a final note, adults found it tough in the workshops to switch to the student mind frame and let go of their existing knowledge and experience, but once they did it, they enjoyed the Learning Scenario design as much as if they were students.

Breakout Group 2

University: Teaching to promote knowledge on and implementation of the Three Rs


Photo: © Anna Persson


Photo: © Fredrik Törnqvist


Photo: © Charlotte Nilsson

Annika Hanberg and **Elin Törnqvist,** of the Institute of Environmental Medicine at Karolinska Institute, a medical university in Stockholm, Sweden, reported back on the breakout session on university-level teaching. The session was co-hosted with **Charlotte Nilsson** from the Research Institutes of Sweden (RISE).

Prof Hanberg said they had shown some interactive examples of how the Three Rs are taught in the Toxicology Master's (ToxMaster) programme at the Karolinska Institute, as well as material from an upcoming documentary film following the process of students' learning of the Three Rs.

Dr Törnqvist described how participants in the breakout session were asked to do a Mentimeter real-time voting survey, which is one of the tools used to teach the Three Rs in group exercises and workshops at the university. This started with a word cloud of Refinement, where participants wrote examples of how to minimise pain and suffering and how to improve animal well-being. They were then asked to rate which methods and strategies they thought contributed most to Reduction of animal use: Computer models; Cell models; Collaboration between research groups; Biobanks; or Statistics and Study design. Finally, they were asked for examples of Replacement.

We believe that the students need to learn and experience all the Three Rs, Replace, Reduce and Refine to understand the Three R concept and to be able to be part of the development towards replacement, she said.

Prof Hanberg summarised the outcomes of the breakout group. We have seen a substantial development of both the extent and quality of the Three R teaching in the Karolinska Institute's ToxMaster programme during the last decades, she said. In the current curriculum, Three R training runs like a track throughout the programme. Open discussions among students are of vital importance for their learning.

We believe that it is very important to include all the Three Rs in our teaching to get an overall perspective on both animal and non-animal methods, as well as to prevent any polarisation between groups using different methods. It is also clear that promoting the Three Rs goes in line with increased scientific quality.

Success factors at our ToxMaster programme are our engaged teachers in combination with regular inspiring visits to the JRC and ECVAM, she concluded. And, of course, the engaged students who will be the next-generation toxicologists and who bring their knowledge, skills and attitudes to the wider society!

Breakout Group 3

PhDs/young professionals: JRC Summer School, how to build an AOP

Photo: © Elisabet Berggren

Elisabet Berggren and her colleagues from the European Commission's Joint Research Centre (JRC), introduced participants to an interactive learning module, called 'How to build an Adverse Outcome Pathway (AOP)'. This module forms part of the teaching programme at the JRC Summer Schools for young professionals.

In the 'escape room' scenario participants help Adelaide, who has locked herself into her office. There are lots of threats and stressors out there! By answering a series of questions and completing various puzzles, the breakout group participants helped her construct an AOP, from molecular initiating event, through cell, tissue and organ responses, to the adverse outcome. As a result, Adelaide escaped from her office, and felt more confident about finding new approach methods to better protect herself and others from all the stressors.

Dr Berggren said that it was harder doing the module virtually than it would be teaching face-to-face at the Summer School. We had interactions with breakout group participants via SLIDO, she said. From these surveys, we learnt that we had a very knowledgeable group of people in the classroom! We were doing the lesson on how to construct an AOP, so there were no differences in opinions or ethical arguments. We also had a question section at the end of the module.

5 discussion points

- *Training in alternative methods requires a multidisciplinary skillset. A good start would be an understanding of molecular or physiological mechanisms.*
- *Tools like MOOCs are best co-created with teachers, who know what works in the classroom.*
- *Flexible teaching programmes can cover academic and non-academic education at different levels, though care may be required for young children.*
- *New technologies like Virtual Reality have great potential for training.*
- *The speed and extent that alternatives replace live animals will depend on education stage and the skills to be taught.*

Session 3

CUTTING EDGE SCIENCE: LATEST SCIENTIFIC ADVANCES TO IMPROVE RESEARCH AND TESTING TOOL BOX

This session focused on scientific advances that are replacing or reducing animal use in science, in particular, physiologically based kinetic (PBK) models; *in-silico* methods and data reuse; Organ-on-Chip technology; *in-vitro* assessment of developmental toxicology; 3D human tissues for drug testing; and non-animal alternatives in biomedical research.

The contributors were: **Ans Punt** (Wageningen Food Safety Research); **Maddalena Fratelli,** (Mario Negri Institute); **Peter Loskill** (Eberhard Karls University Tübingen); **Giel Hendriks** (Toxys); **Christodoulos Xinaris** (Mario Negri Institute); **Francesca Pistollato** and **Laura Gribaldo** (JRC); **Erwin Roggen** (ToxGenSolutions); **Anne van der Does** (Leiden University Medical Center); and **Joan Montero** (Institute for Bioengineering of Catalonia).

Gaining confidence in physiologically based kinetic (PBK) model predictions for quantitative *in-vitro* to *in-vivo* extrapolations

Photo: © WFSR/Ans Punt

Ans Punt
(Wageningen Food Safety Research)

Ans Punt from Wageningen Food Safety Research (WFSR), a university-based research institute in the Netherlands, focusses on the use of kinetic models to predict human blood concentrations and relate these to *in-vitro* biological effect concentrations.

Normally, the starting point is an animal study, she said. Dose levels are identified that do not induce significant effects. Then a safety factor is applied, typically a factor of 100 for humans to be on the safe side.

If you switch to alternatives, like *in-vitro* toxicity data from cell cultures, the extrapolation step can be quite difficult. This is because biological effects also depend on how much chemical actually enters the body, and the blood and tissues. To take this into account, you can combine *in-vitro* tests with computer models that simulate internal concentrations of chemicals in the body, explained Dr Punt.

These computer models, called physiologically based kinetic (PBK) models, therefore enable the replacement of animal tests. The process of converting *in-vitro* effect concentrations into biological active doses in organisms using PBK models is called 'quantitative *in-vitro* to *in-vivo* extrapolation' (QIVIVE).

To illustrate this in practice, she summarised recent work by her team on four structurally-related food additives: methylparaben, propyl gallate, octyl gallate and dodecyl gallate. In particular, propyl gallate is used as a preservative in vitamin D droplets for babies and small children. There is increasing concern about the use of these additives, but gaps in the animal toxicity data limit their full evaluation. Therefore, the team set out to see if *in-vitro* data and computer modelling could provide answers.

A range of *in-vitro* toxicity data and potencies exist for these four compounds, for example in the EPA ToxCast database. All four are estrogenic, mimicking the estrogen hormone, but only at very high concentrations. A key question is whether these effects are relevant. The WFSR team identified a more interesting effect in the ToxCast data - an inhibitory effect of propyl gallate on thyroid peroxidases (TPO), an enzyme involved in thyroid hormone homeostasis, at relatively low concentrations (0.6 µM). We wanted to know if these effects could happen in the body at physiologically relevant dose levels, she said.

We developed PBK models for the four compounds, explained Dr Punt. These incorporated liver metabolism and passive uptake rates based on *in-vitro* data. We used computer (*in-silico*) models to calculate partition coefficients and these reflect, for example, how a chemical sticks to fat tissue. All these processes together in a model simulate blood concentrations.

All the models developed were assembled into an online toolbox[20] that everyone can use. We are also giving courses on this in April 2021, she said.

For all four chemicals, extrapolations were made to oral doses that would be needed to cause adverse estrogenic effects. It was concluded that use levels of all four chemicals were far below these dose levels.

For propyl gallate, the team predicted that you would need a dose of about 6 mg/kg body weight before you reached the TPO effect. In this case, exposure is relatively close to the dose level that could inhibit thyroid hormone activity. Therefore, they concluded that propyl gallate is a priority chemical requiring further studies to explore the effect on TPO.

I do not see this as a direct replacement of developmental toxicity studies, clarified Dr Punt, but as a prioritising or screening tool. More complex *in-vitro* studies are needed to study this effect of TPO. You can also see it as a refinement or a reduction process in animal toxicity studies. Technically, propyl gallate will need testing in animal studies to get additional information on TPO effects, but this screening removes the need for animal testing for all the chemicals in this group.

20. http://www.qivivetools.wur.nl/

This example shows how important this extrapolation step is, she said, to understand how doses lead to effects. For many chemicals, you can make predictions of plasma concentrations based on this type of input data, but for some chemicals you need more advanced models, for example, that take into account metabolism or transporter processes.

A key question is, how good are these models? If you move towards alternatives to animal testing it is really important that you gain confidence in their predictions. We are working on further projects to gain confidence in PBK model predictions, she said.

One project, a pre-study for OECD for liver metabolism, looked at a range of chemical studies in the literature. The team found up to hundred-fold and even thousand-fold differences for *in-vitro* liver clearance measurements between different researchers. This showed the assay was not yet ready to be used by decision-makers. They are now looking at improving standardisation and reducing the variability between labs.

In another project, a private-public project with BASF and Unilever, the WFSR team is looking at predicted plasma concentration values of PBK models with observed plasma concentration in humans. For over 40 chemicals, a huge variability in outcomes was found. Some chemicals cannot be adequately predicted in this way, and the team are looking at the characteristics of these chemicals.

Overall, I would like to conclude that physiologically based kinetic models are crucial in non-animal testing strategies, and that standardisation of the procedure to derive *in-vitro* input parameters is needed to gain regulatory confidence in the model predictions, said Dr Punt.


Photo: © Martina Maiello

How *in-silico* methods and data reuse can help reduce the number of animal experiments

Maddalena Fratelli
(Mario Negri Institute)

Maddalena Fratelli of the Pharmacogenomics Unit at the Mario Negri Institute in Italy noted the wealth of data being generated by high throughput analytics. For example, the Cancer Genome Atlas (TCGA) has molecularly characterised over 11 000 cases of primary cancer, and made this available to researchers.

Computational tools are required to analyse all this data, because traditional statistics cannot handle big data on this scale. They also give us lots of new opportunities, she said.

One example is machine learning and 'omics data (e.g. genomics, transcriptomics) to predict drug sensitivity. The goal is to select the diseases and individuals who will benefit most from drug treatment, for example, using biomarkers for drug resistance or drug sensitivity (personalised medicine). Our case used 40 breast cancer cell lines to determine all-trans retinoic acid (ATRA) sensitivity, added Dr Fratelli, and that is one pillar of our analysis. ATRA has anti-tumour activity for some breast cancers.

The second pillar is the gene expression data available for these lines, she said. We used machine learning to identify a list of 139 genes from the Cancer Cell-Line Encyclopaedia (CCLE) association with ATRA sensitivity or resistance.

We wanted to make a generalised cancer prediction model, and used data from the TCGA for cancer patients to build co-expression networks for each of the 33 cancer types available, she explained. We looked for co-expressions present in the majority of cancers and constructed a model based on 21 genes that we hoped would be valid for all cancer types; in effect using ATRA21 for pan-cancer prediction.

The predictions were validated against data available from the GDSC (Genomics of Drug Sensitivity in Cancer) database, reporting screens of one thousand cell lines with hundreds of compounds, including ATRA. The correlation is quite striking, said Dr Fratelli. The same predictions were applied to tumour samples in the TCGA, for example for a sub-type of acute myeloid leukaemia which is now treated in clinics with ATRA.

Dr Fratelli then discussed a clinical trial on breast cancer that had been approved based on their work, which did not require any animal testing. In this case, a window of opportunity exists between biopsy/diagnosis and surgery (about a month). During this window, estrogen-receptor positive breast cancer patients are treated with ATRA in the neo-adjuvant treatment (a first step to shrink a tumour be-

fore the main treatment). This was aimed at confirming the efficacy of the ATRA treatment and at evaluating the diagnostic potential of a gene-expression model to predict sensitivity to this drug in particular patients, she said.

I think personalised medicine is a good challenge because we are forced to try and find alternative ways of confirming hypotheses, as it is very difficult to develop animal models that recapitulate and describe disease originating in humans. This is a good opportunity, said Dr Fratelli.

Another example from the Mario Negri Institute (from the team of Emilio Benfenati), is the prediction of molecular properties using QSAR (Qualitative Structure-Activity Relationships). Based on the properties and bioactivity of known molecules, QSAR predicts properties, such as toxicity, of new molecules. This is especially important in sectors like cosmetics, where regulation is no longer based on animal toxicity studies. The QSAR models are freely accessible on the VEGA HUB (www.vegahub.eu). This proB vides 80 models that predict human toxicity, eco-toxicity, and other properties.

Dr Fratelli also mentioned the EOSC-Life project, which brings together 13 Life Science research infrastructures to create an open, digital and collaborative space for biological and medical research. The project publishes FAIR data resources that can be reused on the European Open Science Cloud (EOSC).

A strand in their work regarding data reuse is a review of pre-clinical studies leading to clinical trials in ovarian cancer. The mean number of authors with computational expertise on each of the publicly available pre-clinical study papers was 5.8, indicating that a lot of expertise in this area is required.

In summary, Dr Fratelli highlighted the following opportunities for *in-silico* methods: i) to predict classes of patients responding to a given drug, for instance using multi-'omics data; ii) to predict drug safety/toxicity using chemical and target gene information; iii) to model optimal drug combinations (e.g. taking into account synergistic actions); iv) to use more datasets to improve the external validity of the studies.

She also noted that there was a need for more computational biologists, and more 'cultural mediators' with the biomedical community to improve dialogue; and also a higher availability and interoperability of data from different fields.

Peter Loskill
(Eberhard Karls
University Tübingen)

Speeding up the development of Organ-on-Chip technology

Peter Loskill, Head of the µOrgano-Lab of the University of Tübingen and the Fraunhofer IGB, Germany, introduced Organ-on-Chip technology. He stressed that it is a multidisciplinary endeavour, bringing together expertise in engineering (micro-fabrication, microfluidics) and biomedical science (tissue engineering, stem cells/organoids).

The most salient features are: i) human tissue, ii) with *in-vivo* structure and function, iii) in a microphysiological environment, iv) which features vasculature-like perfusion. A key aspect of Organ-on-Chip (OoC) systems is that they are fit-for-purpose. This means starting with your research question and endpoints in mind, and knowing your tissue structure and function, to design your Organ-on-Chip, he said.

The next step is implementation, taking into account cell sources, biomaterials (e.g. hydrogels, scaffolds), chip materials and fabrication methods, perfusion (e.g. flow rates, oxygen/nutrient supply), and sensor and actuator integration. You end up with an OoC-model, which requires functional validation (e.g. case studies, training compounds) to obtain a proof-of-concept. Only when you have enough confidence can you use your model as a fit-for-purpose application.

The most obvious application is pharmaceutical R&D, said Dr Loskill. Organ-on-Chip models can potentially be integrated throughout the entire drug development pipeline, from drug discovery, through the pre-clinical and clinical phases, and even as companion diagnostics after approval. Already today, there is an increasing adoption of these models by pharmaceutical companies.

A key reason for this are cost reductions: the EU H2020 ORCHID (Organ-on-Chip In Development) project team found that stakeholders expected a reduction in costs of about 10-26% for the drug development process (roughly $50-$170 million per drug). Costs are being driven down by success rates. Organ-on-Chip screens out drugs earlier, with less drugs failing at a later stage and only the most promising moving to the clinics.

Besides adding these new models to the current pipeline, Dr Loskill also proposed an alternative approach of redesigning the entire pipeline based on microphysiological systems (MPS), to have a human-relevant pipeline from start to finish. This would encompass organoids and Organ-on-Chip, through to complex multi-organ-chips, using computational approaches to correlate all the data, for example, with a particular patient cohort or individual patient-specific induced Pluripotent Stem Cells (iPSC) as a step towards personalised medicine.

Organ-on-Chip is, however, more than just a drug development tool. Future applications could also include toxicity screening, disease modelling, clinical research, and mechanistic biomedical studies.

Dr Loskill gave some examples of Organ-on-Chip model development in the µOrgano-Lab: Eye-on-Chip, Breast Cancer-on-Chip, Pancreas-on-Chip, Cervix-on-Chip, Heart-on-Chip, WAT (white adipose tissue)-Liver-on-chip, Adipose-on-Chip, Cartilage-on-Chip.

The first Eye-on-Chip model developed was a Retina-on-Chip, a complex system with interacting cell types in a layered configuration. To achieve this we combined organoid and Organ-on-Chip technology, he said. Organoids comprise the most relevant cell types in physiological layering, but are missing key aspects. Therefore, we added a chip platform to provide vasculature-like perfusion and epithelial components. He noted that photo-receptors grow out segments and interact with the retinal pigment epithelial layer, which is a key aspect of the functionality of the retina.

A current limitation of Organ-on-Chip systems is that they are complicated to use, and are low throughput, noted Dr Loskill. That is why we are also working on enabling technologies, to make them more scalable and easier to use. One of these enabling technologies is the Organ-Disc.
An Organ-Disc is based on disc-shaped polymer units which have cell channels, membranes and media channels. Channels for cells and media run radially from the inside to the outside, branching as they go. Cell channels have tissue chambers at their ends (around the outer edge of the disc) which are fluidically connected to the media channels via the membrane. A cell suspension is added to the cell channel inlets in the centre of the discs, which is then rotated at a precise speed to transport the cells into the tissue chambers where they grow into dense 3D tissues. Multiple media delivery can be used to perfuse all the tissues at the same time with different treatments.

We can also use this Organ-Disc concept to analyse tissues sequentially, by stepwise rotation of the disc under a microscope or other sensor, he explained. You can also do multiple loadings for more than one cell type to generate well-defined layered tissue structures.

Looking at the wider picture, the main aims of Organ-on-Chip are to diminish the need for animal testing and make pharmacological/toxicological research more human-centric. However, to achieve this there are still big challenges to overcome, one of which is the so-called 'valley of death'. On one side, academics and start-ups develop the chips, but are not interested in screening large numbers of compounds; on the other, industry deals with large reference datasets and automated handling.

Dr Loskill listed some mechanisms to help bridge this gap between academia and industry: focused funding programmes; earlier regulator involvement; tissue chip testing centres; standardisation; tailored training programmes; and initiatives from EUROoCS (European Organ-on-Chip Society).

EUROoCS has around 500 members from all over Europe. It has the goal of fostering interdisciplinary and inter-sectoral collaboration to promote the development of OoC technology, in line with the Roadmap for Organ-on-Chip technology Europe developed in the ORCHID project.
Training is a very important part of this, but is often overlooked. Novel and complex models require new types of training in multidisciplinary programmes, for developers, end-users, regulators and policymakers, concluded Dr Loskill. Education can start earlier, of course, and we also recently produced an introduction for kids on this subject.

Microphysiological systems (MPS):

Organ-on-Chip:
a microfluidic device, containing living engineered organ substructures in a controlled microenvironment, that recapitulates one or more aspects of the organ's dynamics, functionality and (patho-)physiological response in-vivo under real-time monitoring.

Organoid:
a 3D multicellular in-vitro tissue construct self-assembled in the process of (adult or pluripotent) stem cell differentiation that mimics tissue structure and function.

Moderated discussion

Teri Schultz *(co-moderator): Where do you hit resistance?*

Peter Loskill *(Eberhard Karls University Tübingen): I don't think there is one point of resistance. It's a multifactorial issue. The key is gaining confidence in new models, to move away from the established mind-set of thinking you have to do animal studies.*

Maddalena Fratelli *(Mario Negri Institute): On gaining confidence and understanding, new methods involve difficult things to understand. The second thing is that we need time to demonstrate the soundness of the results.*

Ans Punt *(Wageningen University): I agree, but it also relates to chemicals that are already heavily regulated because of certain effects. I do think you need to see that in your in-vitro data. As soon as decision-makers see chemicals appearing negative in an in-vitro toxicity assay, where they should have been positive, the confidence is gone. So you need to focus on showing that it works in-vitro, including for more difficult chemicals.*

Joris van Meenen *(University of Antwerp): Are shear stress measurements feasible with the Organ-Disc?*

Peter Loskill: *Yes, one of its key aspects is that you can change the flow rate, over a very wide range with a very high precision and generate shear forces over the entire physiological range.*

Arianna Giusti *(Cosmetics Europe): With the Organ-Disc, could you also check metabolism, for example? For which application is it more suitable?*

Peter Loskill: *The Organ-Disc is a platform technology, an enabling technology, and what you can model is down to the tissue that can be generated in it. Checking metabolism is one of the interesting aspects of Organ-on-Chip to look at. We have looked at adipose tissue and combining that with, for example, liver models where we explicitly try to understand metabolism in a human-centric way.*

Denise Bloch *(German Federal Institute for Risk Assessment): What is the status of PBK models for complex mixtures, e.g. plant protection products?*

Valeska Stephan *(UM Rostock/DFG Commission on Animal Protection and Experimentation): What input data are the PBK models based on? Is it human data?*

Ans Punt: *Yes, you can use human data, for example, liver material obtained from surgeries or diseased patient, to do metabolism studies. Other input parameters are calculated from molecular characteristics, like how good a chemical sticks to fatty tissue. It can all be based on in-vitro and in-silico approaches, without live animal studies.*

Complex mixtures are challenging, but feasible within PBK modelling. Simple mixtures are easiest, you can make models that integrate two chemicals and have interactions. This is common practice already in pharmacology for known drug-drug interactions, which could be extended, for example, to plant protection products.

Nadia Wenske *(Perstorp Group): Is there a standardised tool or set of criteria to assess the reliability of different in-silico studies for optimal use in Weight-of-Evidence Methodology / Systematic Review?*

Maddalena Fratelli: *Not that I know of. I think we are still exploring the field and there are many approaches. We try to have validation in different data sets and devise ways for the model to be valid independent of the conditions. I would not say there is a standard now.*

Freya Jay *(Universitätsklinikum Freiburg): How many animal-derived components are still needed to build an Organ-on-Chip model, e.g. for the in-vivo structure (collagen) and the perfusion (growth factors)? And are animal experiments still needed to improve this technology?*

Peter Loskill: *This is not just relevant for Organ-on-Chip, but to the entire field of cell culture. There is a huge amount of animal components used in cell culture. The good thing is that Organ-on-Chip can use well-defined media, synthetic compounds and human serum if necessary, for example, and hydrogels (3D network of artificial hydrophilic polymers) as a support structure. The reason we use a lot of animal compounds in cell culture is because we have a single cell type which has to be cultured in a non-physiological manner, without factors supplied by other cell types.*

The increased complexity of the system with Organ-on-Chip helps move away from animal use in its entirety. We do not need to run animal models to confirm it. This is also a mind-set question, because we do not want to replicate everything that has been done in an animal, we want to replicate what is happening in humans. If we already know that animals do not represent humans very well, why do we have to compare our models to animal models?

Patrik Milić *(Blood Transfusion Centre of Slovenia): There are quite huge gaps between in-vitro models and the functioning of live mammal tissues. At which time point could we say that Organ-on-Chip could replace animal testing?*

Peter Loskill: *The question is not, you have an animal study here and you are going to replace that one by one with an Organ-on-Chip model, it is not a direct replacement, it is a rethinking of the approaches. In the end, it might not be an Organ-on-Chip*

that replaces an animal study, but a combination of Organ-on-Chip with in-silico methods that in the end give us a very different type of data than an animal study would have given us. We will never have the complexity of an entire animal, but we have human tissue and this combined with an in-silico model might be better than a complex animal model. The timeline is tough, this depends a lot of the speed of development and funding in the field.

Ans Punt: *I agree, we are not going to replace one-to-one, it is about creating a toolbox. The efforts should capture the rate-limiting steps and capture mechanisms. It may even be better data than with animal studies.*

Maddalena Fratelli: *Any model is approaching reality but is not reality. You can approach reality from different points-of-view and different combinations of models.*

John Atkinson *(UCB Celltech): Are the model parameters based on healthy volunteers? Do you adjust the parameters for pharmaceutical testing – for instance will renal clearance be altered in a Chronic Kidney Disease patient?*

Ans Punt: *In Pharma, it is common practice to alter the parameters to the population of interest. So they could be changed, for example, to obese or renal deficient. In toxicology this is less common, as the focus is on general population differences, but there are possibilities to simulate subgroups in populations.*

Zeynep Erdem *(Austrian Agency for Health and Food Safety): For us risk assessors, the question that comes to mind regarding Organ-on-Chip technologies is, where does this fit to the assessment strategy? For example, can such a study predict also systemic effects, such as bodyweight changes? What are the limitations? Such developments should also go hand-in-hand with the authorities and the exact application should be worked out together, specifically endpoint-tailored.*

Peter Loskill: *The key question is where does it fit in the strategy, and I think this is the wrong starting point because we have a strategy based on traditional models, maybe we have to rethink the strategy. The more specific answer is at systemic level.*

With Organ-on-Chip technology you can connect multiple organs with each other, you can look at systemic effects. I think the most important aspect is doing that in close collaboration with risk assessors.

Gabriel Sollberger *(Max Planck Institute for Infection Biology): How accessible are these organs, i.e. could you access the specific cell types after treatment for 'omics analyses rather than microscopy?*

Peter Loskill: *One of the downsides of Organ-on-Chip is that you are usually working with a small amount of cells, so your endpoint analysis has to be doable with only a few cells, but most of the models are accessible so you can get your cells out for molecular analysis.*

Martje Fentener van Vlissingen *(Erasmus MC): Obtaining human tissues presents ethical and logistic challenges, as well as considerations about what individual patient information can be released with the specimen. How to tackle these?*

Ans Punt: *I buy mine off-the-shelf, so this is not a direct issue.*

Peter Loskill: *We have two different approaches. One approach is focusing on induced pluripotent stem cells (iPSC) and this is a huge field where a large number of cell lines are already commercially available. The other approach concerns clinical data from patients with personalised treatments, and this goes through the standard ethical committees of different bodies.*

Maddalena Fratelli: *In EOSC-Life we work on sensitive patient data/samples.*

There is an open access book by the European Research Infrastructure for Biobanking (BBMRI-ERIC) 'GDPR and Biobanking[21]' that provides ways to tackle this challenge. I think privacy issues are very important and we should address them, but they should not impede research work.

21. https://link.springer.com/book/10.1007%2F978-3-030-49388-2

ReproTracker: a human stem cell-based biomarker assay for the *in-vitro* assessment of Developmental Toxicity

Giel Hendriks
(Toxys)

Giel Hendriks, CEO of Toxys, a biotech company in the Netherland that provides *in-vitro* toxicity screening solutions, focused on understanding the mechanisms underpinning toxicity testing to replace animal use.

He started his presentation by quoting data from the 2020 European Commission report on animal use for scientific purposes[22]. For the 8.95 million animals used for research and testing (2015-2017), the top use was for reproductive toxicity, and the fourth highest was developmental toxicity. In these areas, there are few well-validated alternatives to animal testing. One reason is the complexity of the reproductive cycle of humans and mammals, he said. For that reason, we focused on one stage in our technology, prenatal development, covering embryonic development from its earlier stages.

We started with human induced pluripotent stem cells (hiPSC) and the differentiation of these stem cells into the tissues that make up an entire embryo, explained Dr Hendriks. They developed and optimised *in-vitro* protocols to get pluripotent stem cells into different mature tissues, such as mature liver-like cells (hepatocytes), heart-like tissue (cardiomyocytes) and neural tissue, based on changes of media and additional growth factors.

We identified a range of biomarker genes that show the effectiveness of differentiation, or indicate the degree of disruption caused by exposure to certain chemicals. The ReproTracker assay was developed to test compounds for the disruption of early embryonic development, starting from pluripotent stem cells (hiPSC) through to its differentiation into three different mature tissues: via endoderm to liver cells, via mesoderm to cardiomyocytes, and via ectoderm to neural tissue.

The first thing that is very important is the selection of the right doses, he said. We typically select the compounds that are just below the threshold of cytotoxicity. The second stage is to develop hiPSC in 24-well plates into the different tissues while being exposed to different concentrations of the test substance. Cell morphology is measured by microscopy. Toxicity is recorded at set times, with samples taken for RNA isolation and real-time PCR to monitor expression of the different biomarkers.

Validation uses known reprotoxic compounds, for example thalidomide, at a range of concentrations compared to a solvent control. This drug caused clear morphological changes and disrupted expression of the various biomarkers, and was classified by the assay as teratogenic (causing malformations to an embryo or fetus). The assay was initially validated using a list of 15 teratogenic and non-teratogenic compounds that were previously selected for validation of the mouse embryonic stem cell test (mEST). Overall predictivity was around 80%, with the most potent compounds *in-vivo* also showing the highest potency in the *in-vitro* assay.

The validation was extended using 65 compounds, based on ICH guidelines for reproductive toxicity testing produced by the European Medicines Agency. Most of the *in-vivo* positive compounds were also classified as teratogenic in ReproTracker based on the disruption of either liver or heart differentiation. Sensitivity was calculated as 90%. A few of the negative compounds on the ICH list tested positive in the *in-vitro* stem cell assay, so specificity was a bit lower at 77%. However, nearly all *in-vivo* studies were done in rodents, and this is compared with *in-vitro* human stem cells, which in some cases behave differently for reproductive toxicity.

Validation so far suggests very good predictivity for accurate classification of teratogens; though there are potential challenges due to inter-species differences. The assay can be used for early developmental toxicity screening, and the potency ranking of compounds that are in development.

Overall I think the predictivity is quite remarkable, concluded Dr Hendriks. The ReproTracker assay is an innovative hiPSC biomarker-based assay for screening developmental toxicants, based on different stages of embryonic development from primordial layers into mature tissues, which provides insights into molecular mode of action and key events, he said.

22. https://ec.europa.eu/info/sites/info/files/com-2020-16-f1-en-main-part-1.pdf

3D human tissues for drug testing and *in-vitro* disease modelling


Photo: © Christos Xinaris

Christodoulos Xinaris
(Mario Negri Institute)

Christodoulos Xinaris, Head of the Laboratory of Organ Regeneration at the Mario Negri Institute in Italy, talked about using patient-specific human tissue to study disease and develop ***in-vitro*** human tissue/organoids for regenerative medicine and drug testing.

One disease under study is autosomal dominant polycystic kidney disease (ADPKD), caused by mutations in PKD1 and PKD2 genes, said Prof Xinaris. This inherited condition causes small fluid-filled sacs called cysts to develop in the kidneys. There is no current cure, mainly due to mechanistic complexity and huge phenotypic variation between patients. Engineering patient-specific tissues *in-vitro* would advance the study of this disease, improve the testing of drugs, and provide more efficient and personalised therapeutic approaches.

To do this, 3D printed scaffolds were optimised for the conditions allowing cyst-derived cells from a PKD patient to form into polycystic tubules. These were used to test a range of drug treatments. In this way, two treatments were identified as most potent, reducing both cyst size and number in the human-engineered tubules. These two treatments were selected for further study and validated *in-vivo* with PCK rats (a model for polycystic kidney disease). Today, we start with human tissue and validate *in-vivo*, but ten years ago we would have started with a lot of animals and then gone to humans, he said. This represents a significant reduction in animal use.

Another example described by Prof Xinaris is the engineering of human renal tubules from induced pluripotent stem cells (iPSC). A patient came to our Centre of Rare Diseases with a very rare mutation in the Pax2 gene and local glomerulosclerosis, he said. We isolated blood cells and reprogrammed these into iPSC. From these, we studied the development of renal tubules, and found the tissues from this patient had a much reduced capacity to branch. This was corrected with gene editing, illustrating how you can use this system to study developmental defects.

In a further example, *in-vitro* techniques were used to obtain patient-derived erythropoietin (EPO)-producing kidney cells (via differentiated iPSC) that could be transplanted under the skin of a patient. The patient's own kidney cells could no longer produce EPO due to injury, while the available drug is expensive and risky. Prof Xinaris' lab is also aiming to regenerate diabetic heart and kidney tissue by modulating thyroid hormone signalling. To do this, they are developing *in-vitro* models. For the heart model, after differentiating iPSC into cardiomyocytes, they constructed 3D spheroids and cultured them *in-vitro*. These beating spheroids were treated with high glucose levels to mimic hyperglycemic injury. After several days they were treated with candidate substances to modulate thyroid hormone signalling. The most promising substance almost restored the normal phenotype of the organoid, he said.

These examples of regenerative medicine and drug testing show significant advantages over traditional animal systems. However, they also have limitations: (i) organoids can have insufficiencies due to developing in media, leading to anatomic malfunctions; (ii) some organ-specific cell types may be missing, such as neurons, immune cells or vasculature; (iii) tissues may remain immature and have high phenotypical variability; and (iv) tissues may include off-target cell types.

Next-generation methods can overcome these limitations. We will develop more efficient perfusion systems that can replicate more faithfully the *in-vivo* biochemical, mechanical and physical cues of the given tissue or organ. Advances in engineering systems, such as high-resolution 3D printers, could guide growing tissues to differentiate and organise into more realistic organoids. This is an emerging field, where important advances are made each year, concluded Prof Xinaris.

JRC activities: Review of Non-animal Models in Use for Biomedical Research


Photo: © JRC

Laura Gribaldo
(JRC)


Photo: © Emilio Mendoza

Francesca Pistollato
(JRC)

In this session, **Laura Gribaldo** and **Francesca Pistollato,** of the European Commission's Joint Research Centre (JRC), introduced the JRC reviews of advanced non-animal uses in biomedical research, and presented recent JRC activities aimed at defining suitable indicators to retrospectively monitor impact and innovation of EU-funded biomedical research. They then interviewed three scientists who work with non-animal models in three different fields.

Laura Gribaldo outlined how the JRC had started to collect non-animal methods in biomedical research, for fields where most animals were still used. We identified seven areas of research, she said, depending on impact on public health, number of animals used, and the severity of the procedures to animals.

These seven areas are: respiratory tract diseases, breast cancer, immuno-oncology, cardiovascular disease, neurodegenerative disorders, autoimmunity, and immunogenicity of advanced medicinal products.

The first two Advanced Non-animal Models in Biomedical Research reviews have been published, on Respiratory Tract Diseases and Breast Cancer . The other five reviews will follow.

Non-animal methods were collected in collaboration with external experts, explained Dr Gribaldo. We were surprised about the huge number of non-animal models already available in the literature. From our survey, we selected around 300 for our JRC Data Catalogue, which is now publicly available on the JRC Science Hub.

We identified many different end-users for this information, including scientists and academics, she said. The first two reports also have executive summaries for policy-makers. In 2021, we will publish a package of information summarising the reports, including leaflets for a wider audience on all the research areas.

It is important to use different language for communication, concluded Dr Gribaldo, to get the right messages to different audiences, including the general public, patients, NGOs, to share the knowledge and encourage the uptake of alternative methods.

Francesca Pistollato explained how the JRC, in the context of biomedical research, is in the process of identifying a set of indicators to monitor the impact and innovation of EC-funded research.

They have defined 17 indicators clustered into the following 6 categories: funding/economic; dissemination; science and technology; regulation and policy; public and social engagement; and education, training and job opportunities.

The visibility and robustness of the indicators is assessed, leading to data gathering and further analysis. The aim is to identify trends, to show whether we are really reaping the benefits of the money we are investing in biomedical research, she said.

We conducted three case studies to perform this analysis, for Alzheimer's disease and other dementias, breast cancer and prostate cancer; all highly prevalent non-communicable diseases that have been extensively funded over the past 20 years, she explained. In a recent paper , Dr Pistollato and her co-authors identified examples of translational failures in these three disease areas and described how indicators can be used to monitor scientific progress and societal impact of research.

In the long term, we would like to see biomedical research approaches moving towards less expensive and more human-relevant approaches, reducing or even avoiding the use of animals whenever possible, while increasing the chance of translational success, she said.

23. https://publications.jrc.ec.europa.eu/repository/bitstream/JRC118161/final_report_online.pdf
24. https://publications.jrc.ec.europa.eu/repository/bitstream/JRC122309/final_report_online.pdf
25. https://www.mdpi.com/2076-2615/10/7/1194/htm

Laura Gribaldo and **Francesca Pistollato** introduced three guests who work with non-animal methods, and asked them **five questions** about their research.


Photo: © Erwin Roggen

Erwin Roggen, of ToxGenSolutions, works in Alzheimer's disease research. Previously, as Science Manager at Novozymes he was involved for 18 years in the development and implementation of alternative methods to animal testing.


Photo: © LUMC

Anne van der Does is Senior Researcher in the Department of Pulmonology at Leiden University Medical Center, the Netherlands. Her research is on respiratory tract diseases and host defence proteins/peptides using lung epithelial cell cultures.


Photo: © Joan Montero

Joan Montero is Senior Researcher at the Institute of Bioengineering in Catalonia. As a post-doc at Harvard Medical School, he patented a new biomarker for patient responses to cancer. He is now developing personalised cancer therapies using cell-based assays.

Question 1:

What human-based models do you use in your research? And what is the added value of this model in comparison with animal-based approaches?

Anne van der Does: *We use a variety of models in our lab, including air-liquid interface models and organoid technology, and more recently Organ-on-Chip using human lung epithelial cells. We try to fit the models to the research question. In these models we use cells isolated from human lung tissue, epithelial cells but also endothelial cells and fibroblasts.*

I think that is a real benefit compared to animal-based models, in that we go directly into a human system. We isolate epithelial cells from different regions of the lung, and recreate micro-environments, which is helpful when you want to study a specific process.

Erwin Roggen: *The first step is to search the literature to identify what others have done and to build on the existing knowledge. This knowledge is then organised using the Adverse Outcome Pathway (AOP) concept, which is applied in toxicology but now also being introduced in the biomedical area. We use single cell pluripotent stem cells derived from human neurons to identify to what extent environmental chemicals can affect human neurons and trigger sporadic Alzheimer's disease. The next step is a model system where these neurons interact with glial cells, another type of cell known to be involved in Alzheimer's disease development. The third level of complexity is the organoids where most of the cells involved in brain function are present, and where we can challenge them with external chemicals. The last phase, kind of a dream for me, is to eventually have an Organ-on-Chip system where we have a mini-brain, a mini-liver and a mini-adipose tissue talking together so we can get an idea about how systemic risk factors affect the brain.*

What I know is that after 30 years of animal research, we are nowhere in terms of benefit for the patient. It is time to try something different. I cannot know if this is definitely going to work, but I think that with all the technological developments we have had in the past 10 years it is worth trying them out to see if we can break this deadlock the pharmaceutical industry is facing with respect to drug development, and even diagnosis. The failure

ratio in this area is the biggest in biotech, more than 90%. Animal models have not worked, but I am convinced that, given time, these alternative methods might give us hints as to new drug targets, new opportunities and better diagnosis.

Joan Montero: *We work on precision medicine, on personalising cancer treatment. With our assay, we isolate cells from patient biopsy, from the tumour, then we expose them over a short period of time (a few hours) to different treatments/drugs we want to test. We then measure phenotypic events, and this allows us to say if the cancer responds or not to therapy; in other words, a functional biomarker for therapy response. I am currently supervising a small team of people to foster uses of this technology, by implementing different bioengineering tools and we are also working in close collaboration with oncologists and cancer researchers to find better treatments for different types of cancer.*

For personalised cancer treatment, mouse models in particular have played a really important role. Genetically engineered mouse models, especially for Patient-Derived Xenografts (PDXs), have been used to mimic the tumour from patients and test different therapies in-vivo. While these approaches have helped us to better understand cancer biology, however, these animal models present different problems.

They are very expensive, it takes time to get enough mice to test different therapies, and you have to use many mice to get the information you want. For these reasons, the use of animal models for personalised cancer treatment is difficult to implement in a hospital. You cannot use dozens of mice per patient, it is not viable. That is why I think functional assays like ours, and those being developed by others, can play a really important role in fostering precision-based cancer treatment.

Question 2:

Do you apply your models as a stand-alone, or do you use them in a complementary manner?

Anne van der Does: *We usually use them in a complementary fashion, for example, we often use organoid technology for expansion of cells, such as when we isolate alveolar type 2 cells from lung tissue where there is not a lot of them to do experiments. We can seed them in our lung chip or Transwell cultures, so we can combine these techniques to make a cell culture to answer our research question. We also combine our Transwell air-liquid interface cultures with cigarette smoke exposures, combining these things to look at response to smoke and host defence. We do combine models and see how they add to each other, it depends on the research question.*

Erwin Roggen: *Same for me, it depends on what you want to do. We have combined existing knowledge with data derived from single cell cultures based on neurons derived from pluripotent stem cells. We have squeezed these data through big data analysis and clustering to identify relevant processes, gene hubs and biomarkers. These biomarkers will go directly into humans. I don't know if you can call that a stand-alone as such, but it is based on a cell assay and computer analysis and then it goes to humans. On the other hand, this assay is the first step, and the next step is a slightly more complex system with two cell types, then an organoid (brain), and the fourth step is an organoid type approach. All these together will hopefully provide information on which drug targets we could go for in future. If it's diagnostics, it is a stand alone, but if drug development it must be an integrated part of a series of assays that have to be seen as a package.*

Joan Montero: *In my case, where we study cancer and better ways to treat it, it is a complex disease and needs a multidisciplinary approach. We work closely with other researchers and especially clinicians to identify the best treatment for cancer patients. The scenario would benefit from information, from pathologists, molecular characterisation, that enables us to narrow down drugs that we want to test in the functional assay. With this information we can run our assay in 24 hours, and let them know which treatment is more likely to cure that patient. In that sense we all benefit from working together.*

Question 3:

What are the technical limitations still to be solved?

Joan Montero: *We are using functional assays and I think that many other laboratories are using different approaches. One of the problems for functional analysis, especially for cancer treatment, is the access to viable cells. We need to interrogate cancer cells in order to identify if treatment is going to be effective or not to cure a cancer patient. In that scenario, what is really challenging is access to samples. When we work with clinicians and get access to a tumour, we often only have a limited amount of tissue on which to perform this functional analysis. Another problem is transportation. When you have to perform functional precision medicine, if you are close to a hospital you can immediately talk to a clinician and quickly get access to that sample, but if from another hospital you have to figure out the best means of transportation, particularly for solid tumours.*

Erwin Roggen: *Since I am representing a company, I cannot reply for academia, but still for us I think the biggest challenge is to demonstrate that the cells we have in culture are physiologically relevant for what we want to acquire information about in a human context. That is not always easy, for me it is the biggest challenge. Also, for instance, we want to compare 'normal' neurons derived from pluripotent stem cells from healthy persons with those*

from Alzheimer's disease individuals, but it is a challenge to really be sure that these were cells derived from a person who was healthy and not in the process of developing Alzheimer's. Proper stratification of patients is a challenge that may not be so obvious, but is really important if you want to develop a test that will give you information to develop a diagnostic approach or identify drug targets.

Anne van der Does: *One of the things when we go into more complex cultures combining different cell types is we have difficulties finding the right media that suits all the cell types. Epithelium often needs very different factors than immune cells, for example, or endothelial cells. Finding the right set up for healthy co-culture of several cell types is quite a challenge. Another thing, is that we are also still using a lot of plastic, for example, the membranes we use in our cell cultures are plastic-based. We would like to go towards more biological materials at some point. However, even a change in plastics can be quite a technical challenge. When we transformed some protocols from Transwell to PDMS-based membrane, we needed to re-optimise our biology on that material. Lastly, another challenge when moving into more complex cell models, is how to layer different cell-types and get them in the right place.*

Question 4:

What are the hurdles to overcome in your field of research to increase the level of confidence in the use of non-animal approaches?

Anne van der Does: *A lot of people who don't work with this technology, especially Organ-on-Chip, have difficulties imaging how this technology works. I think good communication, training and making protocols workable and less intimidating is important, to make people start working with them. Pre-pandemic, I invited researchers to our lab to look at the platform and to see how it works and it really helps. Communication will show that it is not such an intimidating technology.*

Joan Montero: *For personalised cancer research, for the last 10 years, it has mostly been led by 'omic approaches, by genetic markers especially, and also the use of PDXs, as mentioned before. I think the challenge is to move to new technologies, including new functional assays and to implement them in the clinic; to validate them so they can prospectively guide the clinical decisions and better treat cancer patients.*

Erwin Roggen: *What I very often hear when I talk about non-animal methods, whether in toxicology or the biomedical area, is 'it will never be possible' and I think that it is a very weird statement to make for people who are working in research. There are many reasons why people say that, but the worst thing is that you cannot discuss with them, they are completely locked into their own zone and are not willing to step over the threshold. I think the only way to convince them is to be persistent, stubborn, continue using these methods and demonstrate with data that you can actually acquire a lot of useful information without using animals.*

Question 5:

What strategies and tools would you recommend to foster the acceptance and use of non-animal approaches?

Anne van der Does: *I think promoting networks between research groups working with similar technology, and developing programmes that support and validate models or data derived from these models. Also, supporting in some way the making of more robust models, easier-to-use and more accessible protocols, and more training opportunities.*

Erwin Roggen: *I agree that bringing people together, with different methods and standard operation procedures, is very important, and essential for driving training and technology transfer and to promote the use of the assay or methods. The problem is that it all costs money, and I have not seen any call from the European Commission or anywhere that is specific for this kind of activity. There is no money available for demonstration projects today, nor for training or programme development activities, which would promote real networking (more than a coffee chat), exchange experiences, and create confidence in this area.*

Joan Montero: *I agree, and would say that the key word for me is collaboration. We have to make an effort to collaborate not only among researchers, but also include clinicians, industry, regulatory entities and funding bodies. This is the way to foster new technologies to improve human health.*

Moderated discussion

Teri Schultz *(co-moderator) invites the other speakers and audience to join the discussion. She asked a question about how systematic reviews help regulators and funders identify areas of animal use that not do not predict in humans?*

Francesca Pistollato *(JRC): The problem is that these systematic reviews are relatively few, it takes a lot of time to produce systematic reviews and meta-analysis on the robustness and predictive capacity of any methods, either animal or human based. We need more of those. We need to assess retrospectively what has been done, how or where those models were not useful to reply to certain biological question, and if they are proven useless we should try to replace those with more human-relevant approaches. When this information is available, regulators are more likely to shift away from animal to more human-relevant approaches.*

Erwin Roggen *(ToxGenSolutions): Alzheimer's research is a very obvious example, where genetically modified animals have been used for a number of decades without any significant progress, but it is still very difficult to introduce non-animal methods.*

Janneke Hogervorst *(Universiteit Hasselt): Should we stop animal research on Alzheimer's disease now (considering 30 years of fruitless research) and direct all our efforts to human-relevant models?*

Erwin Roggen: *I think we should stop developing new animal models. We can use what we know from the existing models, to the extent that it fits into what we know from human studies, and use that information, but I do not see the point of developing new mouse models and even less making genetically modified monkeys to get closer to the human system, that is completely absurd.*

Francesca Pistollato: *We have, I think, in the field of Alzheimer's research the highest proportion of genetically modified animal models (mainly murine). It is the biomedical area with the highest effort in this direction, generating brand new last-generation triple or quadruple transgenic models, and these are not really helping to reply to biological questions. I think it is time to do something different. Erwin Roggen's work combining different approaches and gaining mechanistic understanding of disease development, and similar approaches, are the way forward.*

Lindsay Marshall *(Humane Society International): If you did the teratogenicity testing on rat stem cells, would you improve specificity and improve confidence?*
Giel Hendriks (Toxys): We have not tried rat stem cells, primarily for technical reasons, they are not so widely available or established. We did use mouse embryonic stem cells, when we started on the project a decade ago, following a similar approach. I think with different stem cell models you can definitely see differences between compounds. Thalidomide is a good example, where there were differences in mice and human stem cells, where human stem cells replicate much better the effects that are known for people who had exposure. The difficulty with this question is that there is very little human exposure data available. It also has to do with choices. I believe that switching to rat stem cells to replicate the effects in rats is ultimately not the goal we should strive for, it's predicting human effects. We should not be trying to come up with an in-vitro system that replicates the limitations of the animal models, especially for developmental toxicity where there is clear evidence that rats are not a good model for human effects.

Verena Vermeulen *(Geistlich Pharma AG): We have already seen three speakers from the Netherlands. I know that the Netherlands have the defined goal to stop animal testing. Is there special funding or a special research environment for animal replacement research in the Netherlands?*

Giel Hendriks *(Toxys): I wish I could say yes, but I don't think it's the case. There are obviously national agencies supporting scientific research in all areas, including animal alternatives, but I don't think it could be called special funding. Most of the larger projects are funded by the European Commission, e.g. via Horizon 2020.*

Freya Jay *(Universitätsklinikum Freiburg): Is it possible to identify new pathophysiological mechanisms of ADPKD-development with this model to replace mouse experiments with genetically-altered animals, e.g. basic gain-of-function and loss-of-function tests? Or is the main use for this model pharmaceutical testing?*

Christodoulos Xinaris *(Mario Negri Institute): In the model, you can replicate some aspects of the kidney function, not the whole kidney. It can be reproducible and reliable, if you know your target and the mechanism you want to study. If you want to study glomerulus in your system and you do not have a mature glomerulus then your results will not be reliable. In our case, we are studying the size of the tumour, which is the main component of the kidney we are studying in the tubule system. For us this is a reliable model, and we have tested different drugs and identified mechanisms that are altered in this*

specific system. Interestingly, we tested the same things in different patients with different outcomes, underlining the need to personalise even more these kinds of studies because different mutations often give rise to different clinical phenotypes.

***Samantha Saunders** (PETA International Science Consortium): How can we best identify priority areas of regulatory testing for development and validation of new non-animal approaches?*

***Laura Gribaldo** (JRC): I think in terms of validation and qualification of methods in the specific areas of biomedical research, we have to develop concepts on the way towards validation of these models, because we cannot apply the same concept and the same parameters from the past to an assay for toxicological or regulatory purposes. We are here in the environment of basic research, and applied research is completely different, and so we need to develop new criteria to validate these models, to agree these criteria with the academia/scientific community, and start a campaign of funding for validation of new models. We can identify areas of concern in terms of public health, consumption of animals and severity of procedures, and invest efforts there.*

***Stefanie Schindler** (Menschen für Tierrechte): You mentioned that there is a "threshold" in the mind of researchers, like 'in-vitro technology will never achieve', etc. This is actually surprising to hear from people who will always adopt the latest technologies, in the hope that this will add to their science. Do you think there is a psychological barrier? People may have a bad feeling about doing animal experiments, but feel forced to because they are convinced there is no real alternative. They do not want to accept the thought that some things that they did to animals may have caused suffering unnecessarily?*

***Erwin Roggen:** It would be a waste of animal life if we just forget what has been done in the past. I would not say these people have not been doing useful work, after all we have to consider that a lot of mouse studies have contributed to a lot of Nobel Prizes. But, we are at a different stage now, especially for Alzheimer's disease. It has not been possible to break through the barrier with animal models, so it does not make sense to continue that way. That requires a change in mind-set. These people have been earning a lot of money for their research, building their models, so all of a sudden the funding might stop – that could be one barrier. Another way is for researchers to accept that using parts of animals in in-vitro systems, and putting the data together to recreate the animal, or in this case a human, can teach us something. We have to try and go that way, and not see problems but challenges to be solved.*

***Joan Montero** (Institute of Bioengineering): From the perspective of personal cancer treatment, I agree, we have to move to more functional assays to give better chances to cancer patients and reduce the use of animal models for that purpose.*

***Christodoulos Xinaris:** I think that there is a problem of mentality, especially in the scientific community. If you want to publish something, often when you have proof-of-concept in-vitro, a high-impact journal will ask for you to show some animal studies. We also need to be very careful about how we say things in order to change mentality, for example, like 'human brain in a dish' headlines, where reports outstrip actual scientific progress and create false expectations with the public.*

5 discussion points

- ***Networks of researchers who collaborate with clinicians, industry, regulatory bodies and funding bodies can advance non-animal methods.***
- ***Communication can make new technologies less daunting to adopt.***
- ***In areas such as Alzheimer's disease, alternative methods based on a mechanistic understanding of disease development are the way forward.***
- ***Organ-on-Chip technologies require a different strategy than using animal methods, for example, connecting multiple organs to look at systemic effects.***
- ***It is not a 1-to-1 replacement of animal methods, it is about creating a toolbox.***

Session 4

GAINING TRUST IN USING NEW ALTERNATIVE APPROACHES

This session focussed on initiatives that build trust in alternative approaches. Participants heard from two Pharma companies, a consumer goods company and a researcher on changing attitudes to non-animal approaches. Topics included industry's experience with *in-vitro* and *in-silico* methods; quality testing approaches for vaccines using non-animal methods; confidence building approaches for new test methods in developmental neurotoxicity; Organ-on-Chip in drug development; and alternatives in COVID-19 research.

The speakers were: **Ard Teisman,** Janssen Pharmaceuticals; **Shahjahan Shaid,** GSK; **Marcel Leist,** University of Konstanz; **Carl Westmoreland,** Unilever; **Rhiannon David,** AstraZeneca; **Dilyana Filipova,** European Coalition to End Animal Experiments; and **Christian Desaintes,** DG Research and Innovation.

Drivers for the pharmaceutical industry to adopt human stem cell-based models


Photo: © Ard Teisman

Ard Teisman
(Janssen Pharmaceuticals)

Ard Teisman, from Global Safety Pharmacology, Janssen Research & Development, in Belgium, introduced the concept of safety pharmacology, which aims to define the optimal way in which drugs can be utilised without causing harm.

It is a huge challenge to find models that predict for the patient, because there is no such thing as a typical patient, he said. However, despite all the genetic and phenotypical differences, pre-clinical assays need to predict for all patients to be qualified as a good translational model.

Ultimately, proper safety assessment may only be achievable with a set of complementary models representing different physiological aspects, explained Dr Teisman. These models could include human stem cells.

Dr Teisman outlined the main drivers that are leading companies to implement pre-clinical models including stem cell models: (i) good representation of the patient; (ii) secure the safety of patients; (iii) follow guidelines; (iv) do it in a timely fashion; and (v) to support all these aspects there is room to innovate.

The first two drivers put an emphasis on profiling the safety of new compounds. When we bring a new compound to a healthy volunteer or patient, we need to reduce the risk as much as possible by using models that are predictive of effects of humans, he said; a good benchmark is to "only bring those potential new medicines to patients that you would be willing to take yourself."

The guidelines describe the type of assays and profiling required. We are aligned with the ICH (International Conference of Harmonization) Guidelines, he said.

For the drug discovery process, we start with a lot of molecules and use predictive assays to select one compound to move forward to the clinic, explained Dr Teisman. To do this in a timely and profitable manner, we want to use accurate models that, on one hand, do not show false positives because we would throw away potentially valuable compounds and, on the other hand, we do not want false negatives because we invest a lot in compounds and do not want to find at a late stage an effect we missed early on.

Luckily, he said, I work in an innovative company that stimulates 'out of the box' thinking, so we have some room to explore alternative methods. We aim to find the underlying causes of a disease, to effectively target treatments, without off-target effects.

As an example, Dr Teisman introduced the safety pharmacology 'core battery' (ICH-S7A) used to investigate the effects of test substance on vital organ functions, such as the central nervous, respiratory and cardiovascular systems. These organs do not work in isolation, but interact closely to ensure homeostasis. The 'core battery' studies take that into account, though the effects of substances can also be looked at for particular cell types.

He gave a brief history of stem cell cells in regenerative medicine, including the discovery by Shinya Yamanaka of Kyoto University in 2006 that four genes can re-programme an adult cell back to an embryonic state. This work opened opportunities for the use of human induced pluripotent stem cells (iPSC) in biomedical sciences.

At Janssen, a range of iPSC-cardiomyocyte cell lines have been produced to test substances for potentially adverse effects on the heart. The iPSC-cardiomyocytes have a distinctive rounder shape, but beat autonomously like normal heart cells. The collective beating gets disturbed by neurotoxins, which shows an opportunity for these cells to be used in assessing drug induced cardiac rhythm disturbances.

The beating cells can, for example, be used in a calcium ion (Ca2+) transient assay to detect drug-induced 'arrhythmia-like' events. In this assay, cells beating spontaneously are incubated with fluorescent dye to see intercellular activity, enabling a high-throughput imaging analysis. Ca2+ gradients plotted against increasing concentrations of test materials give graphs that align closely to measurements of electrical activity in tissues. The application of reference compounds known to affect the heart, such as digitoxin and veratrine, produces similar changes in action potential in both systems.

Using this assay, cell lines derived from different donors were profiled against 60 reference compounds, having different actions. For example, sodium-channel blockers, Ca2+ antagonists, and those that decrease heart rate, all of which produced measurably different responses in isolated iPSC cardiomyocytes. This enabled the best cell lines to be selected, as they all represent different single donors and behave differently to these challenges.

We see differences between the cells for different providers, so you have to understand your cell line, and understand what your cell line is doing in your model with a large set of compounds, noted Dr Teisman.

He briefly showed an example where the stem cell model (that was thoroughly characterised) replaced animal based models at his company.

Are we ready to implement stem cell assays in drug discovery? Yes, iPSC's can play a role in early safety pharmacology screening, he said. Can we totally replace drug safety screening by only stem cell models? No, and most likely not in the near future. Would I feel safe to take a new drug that was only tested *in-vitro* on iPSC's? No, all models can and should be useful but 100% patient-representative models don't yet exist, concluded Dr Teisman, expressing his personal opinion.

Shahjahan Shaid
(GSK Vaccines)

Consistency approach IMI project Vac2Vac: quality testing approaches for both human and veterinary vaccines using non-animal methods

Shahjahan Shaid of GSK (GlaxoSmithKline) Vaccines in Belgium talked about the Vac2Vac project[26]. The project is part of the wider Innovative Medicines Initiative (IMI) initiative, which with a budget of €5.3 billion, is the world's largest public-private initiative in life sciences research. Within IMI, 15 projects have been launched on vaccines, with a budget of €385 million, one of the largest being Vac2Vac.

Vac2Vac (Vaccine batch to Vaccine batch comparison by consistency approach) represents a paradigm shift, said Dr Shaid. In the past individual vaccine batches were considered unique, but this has changed due to good manufacturing practices and guidelines. Each manufacturing step is now validated with qualified equipment using standard operation procedures, following by quality control (QC) testing.

The project has 22 European partners working together to substitute animal assays for vaccines production. Within the scope of Vac2Vac are 7 vaccine franchises (5 veterinary and 2 human). The project started in 2016 and was due to finish February 2021, though a prolongation has been requested.

The animal assays in the scope of Vac2Vac are 13 QC animal assay substitution targets, covering a range of human and animal diseases (e.g. rabies, diphtheria, tetanus, and blackleg in cattle and sheep). The assays use a range of species, including chickens, mice, hamsters, guinea pigs, and rabbits.

Work to replace these with non-animal assays is structured in four technical work packages: i) physio-chemical methods (e.g. mass spectrometry); (ii) immunochemical (e.g. ELISA); (iii) cell-based assays; and (iv) bioinformatics.

Dr Shaid noted that two of the assays no longer need to be substituted, because they have been removed from the European Pharmacopoeia. The first full success of the project was the completion and approval of the 'Substitute Rabbit pyrogen test for tick-borne encephalitis vaccine (TBEV)' for humans.

Good progress is being made for the other assays, for example, on the 'Rabies *in-vitro* potency assay', through the design of a strain-specific replacement ELISA. A proof-of-concept has been achieved for the 'Substitute *in-vivo* potency assays for Diphtheria, Tetanus and Pertussis', used in both the human and veterinary fields, with the transfer of the methods to industry partners ongoing.

A number of lessons have been learned during the project: (i) a high organisational level is required, to find a common language, align priorities and leverage synergies; (ii) sustainability can be achieved

26. http://www.vac2vac.eu

by defining plans and agreements beyond Vac2Vac to ensure continued lifecycle management; and (iii) a focus on promising tasks by Go / No Go decisions to ensure the transfer of important methods and allow small-scale collaborative study.

The successful collaboration achieved in Vac2Vac is reflected in the 10 completed and 34 foreseen publications, all of them open access, he concluded.

Gaining trust in new ways of assessing developmental neurotoxicity


Photo: © Marcel Leist

Prof Marcel Leist
(University of Konstanz)

Marcel Leist, Chair of *in-vitro* toxicology and biomedicine at the University of Konstanz in Germany, and Director of CAAT-Europe (with Thomas Hartung of Johns Hopkins University, Baltimore, USA), talked about the work of academic institutions who are not just developing tests, but also building trust for their implementation.

To build trust toward achieving regulatory acceptance, both test developers and regulators must be involved. Developers often develop and optimise their methods, but this activity usually has no impact beyond their own community. Therefore, there is a disconnection between the world of test developers and the one of regulators as end users. This needs to be overcome to avoid problems with acceptance, he said.

We need to understand the measures that give regulators trust in new methods and the data they generate, explained Prof Leist. The first thing is to establish reliability, and reduce variations between tests.

Then we need to think of a reference system, which is especially important in fields like predictive toxicology or pharmacology. To exemplify, he described the EU-ToxRisk project, which is building the elements necessary to inspire trust in non-animal methods, document them, and demonstrate them under 'real-life' conditions. The project's aim is to transform toxicological testing by initiating the required paradigm shift towards animal-free safety assessment, based on a mechanistic understanding of adverse effects.

A number of publications (e.g. GIVIMP and ToxTemp documents) contain practical guidance on what a test description should look like, Prof Leist said. OECD is an important source and facilitator for guidance documents on *in-vitro* methods descriptions, data reporting and setup.

The next step is an evaluation of the readiness of test methods for certain applications. Prof Leist presented a rating system to assess if methods are ready for the regulatory acceptance stage. We tried this scoring system for test readiness on a panel of tests in the neurodevelopmental toxicity field, and a general applicability seemed possible, he said.

Test validation methods were tested in a project coordinated by the European Food Safety Authority (EFSA). In this exercise, we tested 120 compounds in different assays to see what was required for building a battery of assays, said Prof Leist. The resulting paper, published in October 2020, set out a protocol for the implementation and interpretation of an *in-vitro* testing battery for the assessment of developmental neurotoxicity[27].

The first step was to demonstrate reliability, with a criteria checklist including robustness and level of understanding of the methods, the handling and identification of test chemicals, data reproducibility from day-to-day/operator-to-operator/lab-to-lab, and knowledge of factors affecting performance/sensitivity. Regulators also want to see this reliability checklist or else trust is not possible, he said.

Then, to move from reliability towards predictivity we need reference compounds, both positive controls (toxicants) and negative controls (no effect). These need to be effectively established and readily available to determine the accuracy and specificity of a method. This can be further improved with a prediction model.

The third aspect is relevance, he said. This can look at the context of IATA (Integrated Approaches to Testing and Assessment) and assays. For example, integration with toxicokinetics to check if assays deliver hazard data that correlates with real-life data. Another exercise would be looking at consistency using structural activity relationships (QSAR) and read across, to predict chemical toxicity based on data from similar, well-studied compounds.

A case study submitted by EU-ToxRisk to the OECD provides such a framing study in the regulatory context, which is as important as the science, claimed Prof Leist. This is what we often forget and this is where for trust building it is important to involve regulators early on.

27. https://doi.org/10.2903/sp.efsa.2020.EN-1938

The study looks at consistency across a battery of existing developmental neurotoxicity assays using twelve valproic acid analogues. Some of the chemicals were developmental toxicants, while some others were known to be non-toxic. The results showed that the battery of methods is quite congruent. If we now fit in a new assay, for example a stem cell assay from our lab, we can see if it fits into this pattern, he explained. If it fits, it again helps in trust building.

It is not just a tick box approach, he concluded, but involves having a number of approaches, which together with experience, builds confidence and trust in the assays.

Case studies of assuring safety without animal testing

Carl Westmoreland, from Unilever's Safety and Environmental Assurance Centre in the UK, talked about their case studies for assuring safety of consumer products without animal testing.

Carl Westmoreland (Unilever)

When we put a new ingredient into a homecare or personal care product, we need to ensure that consumers are safe, he said. Science and risk assessment underpin that safety. Historically, risk assessments depended heavily on data from animals. However, Next Generation Risk Assessments (NGRA) could assure safety without animal testing.

He introduced the SEURAT-1 (Towards the Replacement of *in-vivo* Repeated Dose Systemic Toxicity Testing) project (2011-2016). This EU project looked at how you might assemble available tools into a toolbox, in a decision-making framework for risk assessment. The tools themselves need to be robust, reproducible and transferable, he said, but the project showed that of equal importance is how you put them together within a workflow to make decisions about safety[28]. The current EU-ToxRisk project is building on this earlier work.

Dr Westmoreland then focussed on a case study done at Unilever. This imagined that there was no data for a material frequently used in personal care products, coumarin. The study addressed the question, what would the result of a safety assessment of coumarin be if it was a new ingredient and we used only non-animal approaches?

The non-animal methods safety assessment was done for 0.01% coumarin in face cream and body lotion. It could not draw on any existing data or knowledge derived from animal studies, just the new NGRA framework. The results were published in a paper in 2020[29].

The first information generated was from PBK modelling, to understand plasma levels of the ingredient, he said. Then a tiered approach was used, from *in-silico* tools, to a Safety 44 Screen (activity/binding against enzymes and receptors to show unwanted pharmacological activity); BioMap Systems (immunomodulatory effects); and Stress Pathways (40 biomarkers for 10 stress pathways).

We also added in more global information about coumarin, including data from High Throughput Transcriptomics (HTTr), he explained, where you get a snapshot of all the pathways and genes that the material may have affected in a variety of different cell types using a range of different concentrations.

The conclusion was that coumarin was not genotoxic, did not bind to any of the 44 receptors tested, and did not show any immunomodulatory effects at consumer-relevant exposures. The case study demonstrated the value of integrating exposure science, computational modelling and *in-vitro* data, to reach a safety decision without animal data.

Dr Westmoreland also presented other examples that demonstrate the feasibility of using *in-vitro* bioactivity as a protective estimate of point-of-departure (PoD) in screening-level assessments. We are not trying to predict what the animal study would have shown you, he clarified, but to understand the difference between consumer exposure and the point where you get an alteration in human biology.

Unilever have launched a new surfactant ingredient, called rhamnolipids, in a dishwashing liquid in Chile, under the Quix brand. This is the first example from Unilever of getting a new material onto the market safely without using animal studies to do safety assessments. A bespoke set of NRGA tools was used to do that safety assessment, explained Dr Westmoreland, including detailed consumer exposure studies, *in-vitro* skin penetration assay, a study on understanding metabolism, and *in-vitro* immunotoxicity assessments.

In conclusion, he said, non-animal safety assessments for consumer goods are moving from 'might be possible in theory', at conferences I was going to 5 years ago, to published case studies of NGRA in action. We have shown that you can use these tools to make safety assessments for the types of ingredients and exposures we are looking at in our products.

28. https://www.sciencedirect.com/science/article/abs/pii/S2468111317300464
29. https://pubmed.ncbi.nlm.nih.gov/32275751/

Moderated discussion

Martje Fentener van Vlissingen *(Erasmus MC): Are volunteers or patients on clinical studies mostly aware of the preceding R&D trajectory? And their doctors?*

Ard Teisman *(Janssen Pharmaceuticals): Yes, I think they are made aware. We have to inform healthy volunteers and patients of what is done before they get the medication. There are brochures generated where information is shared with doctors, and they have the responsibility to talk about these things with the patient before treatment.*

Monica Piergiovanni *(Technical Officer, European Commission): In the Vac2Vac table, I see that no methods in the last row (computational) and very few cell-based methods were transferred. Is this due to technical or regulatory reasons?*

Shahjahan Shaid *(GSK): I would say two elements need to be considered. Most of the cell-based assays were not conducted on stable cell lines, as it was more to understand the immune response, and therefore it was difficult to see that this later can be conducted under a Good Manufacturing Practice (GMP) environment. The other element is that a lot of these assays were more difficult to run under quality control testing conditions. However, it is still possible. That is the great thing in Vac2Vac, you have a lot of different technologies that are assessed at the same time and if you know that there is one that gives the same kind of information in a more robust and more reliable way you choose that one.*

Martje Fentener van Vlissingen *(Erasmus MC): Are there also lessons to be learned regarding vaccine development?*

Shahjahan Shaid: *We really have to separate what is a lifecycle product and what is a new product, and also the regulatory requirements are quite different. For lifecycle products, they are highly specific to a given product, for new products they are a bit more general because it needs to be a bit broader. Overall, I would say the technologies are practically transferable to the development of new products, but not fully because a new product is not running under GMP, so more data is needed.*

Monique Janssens *(National Committee for the protection of animals used for scientific purposes): Why would we need still more new ingredients for home care products?*

Carl Westmoreland *(Unilever): There is always the need for improving the products we make, particularly at the moment with a big push for less petrochemical-based ingredients, toward more sustainable and renewable ingredients. There is still a need to make sure those sustainable and renewable ingredients are safe to use.*

Chris Burns *(co-moderator): Thinking 'out of the box', there has to be a mind-set change?*

Ard Teisman: *It is very easy to stay with traditionally acceptable models, but to implement new models that requires people to follow where the science is going. We were following the stem cell research already by 2006, when we started to collaborate with cell providers to try and analyse the value of these. It takes time, but we have the funding to explore that. It is up to the scientist in the field to understand a little bit about the opportunities that are around. At least in my field, I do not hear negativity towards these opportunities. People are well aware of the technologies. However, it requires a cautious approach – we want to understand where the limitations of in-vitro assays are, and there is still a lot of things that can be further improved. We need to have a detailed understanding of our models.*

Marcel Leist *(Konstanz University): It is not always that a mind-set change is required. There are situations where it is natural to use the new approaches. For instance, 20-30% of all the new drugs getting to the market are human proteins, human macromolecules, so you can't even test them in animals. We are really lucky that we have developed stem cell and organoid models now, with lots of papers coming out with artificial blood vessel models, artificial kidney models, Lung-on-Chip models that can be used directly using human tissue and human cells, so the field has already overtaken those that always think in yesterdays.*

Samantha Saunders *(PETA International Science Consortium): The EU cosmetics animal testing ban drove innovation in non-animal methods, but we have since hit a stumbling block regarding tests required under the REACH regulation. How can we bring regulators on board to accept new non-animal methods and recognise restrictions on animal use?*

Carl Westmoreland: *The ban on testing on cosmetics did drive some of the science, and some of the SEURAT-1 project was driven by a lot of that and gave us experience with making those decisions and putting that data together. I think there are really huge opportunities to be able to use science more broadly in other areas where*

toxicology data is used. I think chemical regulation is a key one of those. If you can do consumer safety risk assessment without needing to generate animal data, can that same non-animal data be used in chemicals registration rather than generating traditional test guideline studies? I think that is worth more exploration.

***Teri Schultz** (co-moderator): What drives a company like yours to follow trends on non-animal testing? What are the market-driven reasons to do this? Do consumers read labels if animals are used?*

***Carl Westmoreland:** I have been a toxicologist for over 30 years and I have become a convert to the fact that you can now do a lot of the things we used to rely on animals for without them. Consumers are very aware of this topic. A lot of Unilever personal care brands are accredited as not tested on animals. And that label on the bottles is what people look for. They do not want animal testing for consumer goods.*

***Chris Burns:** So, if a chemical ingredient goes on the REACH list it needs to be removed from products?*

***Carl Westmoreland:** That is a challenge we have at the moment, yes. If animal testing is demanded for chemicals registration and a supplier has to do that, then we lose that ingredient from 'not tested on animals' brands.*

***Shahjahan Shaid:** Regarding business drivers to replace animal testing, I think it is important to understand that animal testing has improved the quality of life of humans for the development of really important drugs. However, we see more non-animal technology arising and industry has an appetite to be innovative and move to this new technology. Then, taking into account the ethical aspect, even people who work with animals in facilities with high ethical standards in Europe, are devoted to replacing them.*

***Marcel Leist:** I think we keep making the same fundamental mistake, in thinking the animal test is the gold standard, and in-vitro methods might not reach this, but this is far from correct. Predictivity of animals is not perfect, and in my experience the failure rate of drug candidates for some diseases is close to 100%. Vaccine development is another example where animal studies often fail. There are certain aspects that can be clarified in mice studies, but the typical side effects of vaccines are immune-system related and will not be predicted for humans using mice.*

***Kirk Leech** (European Animal Research Association): Researchers attempting to develop vaccines against COVID-19 have been heavily dependent on Non-Human Primate models (NHPs). For example, the Pfizer/BioNTech vaccine (the first COVID-19 vaccine approved by the European Commission, on 21 December 2020) relied on pre-clinical data generated by BioNTech in Germany, using rhesus macaques (a species of NHP) to show that recipients of the vaccine were fully protected against the SARS-CoV-2 virus, and to ensure its safety.*

***Ard Teisman** (Janssen Pharmaceuticals): I don't think any scientist would do animal experiments for the sake of it. If they choose animal models, they should have a good idea of how it is useful in the clinic. If not predictive, you should not do the experiment. The flip side is, we have to realise that stem cell models we have currently do not replace animals. I think there is room for further improvement in cell-on-chip models so they have more of a function of an organ.*

Photo: © A.L.S Photography

Rhiannon David
(Astra Zeneca)

Using Organs-on-Chip models to replace animals in drug development

Rhiannon David, Pharmacology & Safety Sciences at AstraZeneca in the UK, talked about the need for microphysiological systems (MPS) in drug development. At AstraZeneca, we are starting to use Organ-on-Chip technology, especially for safety assessment, where it can start replacing animals in some studies, she said.

The drug development process is not very efficient and is very expensive, costing up to $2.5 billion per drug. The pipeline (10-15 years), moves from drug discovery (100 000 compounds), pre-clinical (250), clinical (5), to the regulatory approval of one compound. Over the years, there has been an increasing investment in drug development, but with a decreasing output in terms of successful drug candidates, she said. There are both safety and efficacy reasons for drug failures.

Data from a paper published in 2018[30] showed the success of projects following the introduction of AstraZeneca's five-dimensional framework to improve R&D productivity. This also identified reasons for failures. The five dimensions were: right target, right tissue, right safety, right patient, and right commercial potential.

Though we have seen considerable improvements, there were still incidences of idiosyncratic or unexplained toxicity, said Dr David. Drugs mainly failed due to safety in the pre-clinical phase, and due to efficacy in the clinic phase. This shows us that tools to assess safety and efficacy pre-clinically need to be improved, because the animal models are not always predicting for what actually happens in humans.

Models are needed that better mimic human pathophysiology and improve our understanding of the mechanisms behind the toxicity. That is why we think Organ-on-Chip and other MPS could provide this improvement, she said.

MPS are an improvement on traditional cell cultures in a flask or a dish. They can incorporate features like: (i) multiple cell types enabling cell-cell interactions; (ii) matrices that maintain cell shape and architecture; (ii) models with microfluidics to mimic blood flow; (iv) mechanical forces such as stretch (e.g. in Lung-on-Chip to mimic breathing); and (v) immune components. This means MPS are much more like in-vivo models in recapitulating tissue-level human scenarios.

Therefore, we think MPS can transform our drug discovery process, said Dr David. When we look at the drug development process, we see multiple opportunities for MPS to add value. Early on, to identify and validate potential compounds, using human disease models. At the pre-clinical safety stage, we see real opportunities for human MPS to inform our *in-vivo* design and/or reduce the number of these studies. In the clinic, human-relevant models can be used to follow up clinical findings.

Dr David outlined an example of where they are using an MPS model in pre-clinical safety assessment, in place of *in-vivo* studies. A bone marrow MPS is being used to screen for oncology drugs with bone marrow toxicity, a common side effect of these drugs.

The team are combining different drugs together or in a defined schedule. The challenge is finding the right dose/schedule. This is not something that can be done pre-clinically with traditional cell culture models or *in-vivo* studies. A bone marrow MPS could help fill this gap.

The bone marrow MPS was developed at AstraZeneca in collaboration with TissUse, a Berlin-based company with a unique Multi-Organ-Chip platform. They are using the company's HUMIMIC Chip2 system, which has two independent circuits per chip.

It has three key features: (i) a recirculating fluidics system with two reservoirs, one for sampling the media and one containing the (ii) ceramic scaffold on which mesenchymal stem cell grow in a similar way to *in-vivo* systems; and (ii) a media containing cytokine, to drive stem cell differentiation into erythroid, myeloid and megakaryocyte lineages.

The results, for example, showed a clear exacerbation of toxicity for recurrent dosing, compared to dosing two drugs individually, for the erythroid lineage. This was less so for the myeloid lineage, and absent for the megakaryocytic lineage. The model can therefore detect lineage-specific toxicity (not just broad toxicity).

Looking at the scheduling data, the order certain drugs are given was found to affect the level of toxicity. This highlights the importance of being able to do repeat dosing in these models. The data have provided schedule options for the clinical team to mitigate the toxicity we see when dosing concurrently, she said.

Dr David summarised as follows: (i) bone marrow MPS can recapitulate clinically-relevant lineage-specific toxicity profiles; (ii) extended viable cultures enable repeat dosing to test multiple cycles giving additional information about drug interactions in combinations and schedules; (iii) demonstrable Three Rs benefit for this context of use, giving confidence that for specific contexts MPS can be used as an alternative to animal studies; and (iv) further developments of MPS such as standardisation and real-time monitoring, and patient-derived cells will increase relevance and adoption.

30. https://www.nature.com/articles/nrd.2017.244

Role of alternatives in COVID-19 research


Photo: © Presian Nedyalkov

Dilyana Filipova
(European Coalition to End Animal Experiments)

Dilyana Filipova described the European Coalition to End Animal Experiments (ECEAE) as an umbrella organisation representing many NGOs across Europe, who work towards ending animal experiments. In her presentation, she talked about the crucial roles played by non-animal research methods in COVID-19 research.

One key feature of COVID-19, she said, is that it can affect and damage multiple organs, not only the lungs, but also the heart, brain, small intestine, blood vessels and other organs. Multiple organ infections result in complex symptoms in human patients, and no other animal species can fully recapitulate this.

Many groups are developing safe and efficient drugs and vaccines to prevent the spread of disease and treat patients. However, in general, studies have shown that more than 90% of drug candidates passing all required animal experiments fail in subsequent clinical trials with human volunteers and patients. This is bad for any disease, but especially in the case of COVID-19 where we cannot afford to invest so much time and effort for such a low probability of success, Dr Filipova said.

Therefore, the coronavirus is a perfect example of the need to quickly move away from animal experiments and to move towards implementation and usage of reliable and human-relevant non-animal methods, she said.

Dr Filipova highlighted a few of the many examples of how NAMs are contributing to our understanding of COVID-19 and to the drug development process.

Lung organoids[31] are being used to study SARS-CoV-2 infection in the US. Researchers cultured human lung cells to create alveolar organoids, in which SARS-CoV-2 infection follows the same molecular mechanisms seen in native human lungs. It can be used to analyse treatments and drugs affecting disease progression. The EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) recently published a report, she added, with 284 non-animal models for respiratory and bronchial research[32]. Many of these could potentially contribute to the analysis of COVID-19.

A human brain organoid model, the so-called BrainSpheres model[33], can model infection of human iPSC-derived brain cells with the coronavirus; it was previously used to analyse other viral disease (e.g. Zika, Dengue and HIV). The cells in the organoid express the ACE2 receptor that plays a key role in the infection mechanism. It can be used to analyse virus infection and replication.

Following reports that the coronavirus infects human intestinal cells, a human small intestine organoid[34] was used to study the number of infected cells increasing over time. This was consistent with the gastro-intestinal systems reported by many COVID-19 patients.

The fourth example given by Dr Filipova was the so-called 'heart-in-a-jar' model. This was developed by Novoheart to test the effects of different drugs on cardiac activity. The company recently announced that the model was suitable for analysing the coronavirus for possible cytotoxic effects in cardiac tissue.

A human lymph node organoid, developed by Prellis Biologics, has been shown to produce antibodies against the coronavirus and other viral diseases. This can be used to test antibody therapies against COVID-19. It provides a fast platform, adaptable to continuous virus mutations.

There are many more such NAMs, including organ-on-chip models and *in-silico* models, she noted. A list can be found in the NAT database for non-animal technologies (www.nat-database.org). Some regulatory agencies are recognising the potential of these methods. For example, the United States Food and Drug Administration (FDA) announced a few months ago that it would use human Lung-on-Chip models to analyse the safety of COVID-19 vaccines and therapies.

Dr Filipova concluded by saying that NAMs have a big potential in COVID-19 research, and generally in biomedical research. They have key advantages over animals, being faster and more human-relevant.

She pointed out the need for increased funding and support of NAMs at EU and national level (a study showed less than 1% of the public funds for biomedical reach were dedicated to NAMs); and the desirability of an EU-wide phase-out strategy towards non-animal science (with deadlines and milestones).

31. https://pubmed.ncbi.nlm.nih.gov/33128895/
32. https://publications.jrc.ec.europa.eu/repository/bitstream/JRC118161/final_report_online.pdf
33. https://www.altex.org/index.php/altex/article/view/1924
34. https://science.sciencemag.org/content/369/6499/50

EU H2020 research on COVID-19 and alternatives to animal testing

Christian Desaintes
(DG Research & Innovation)

Christian Desaintes, of the European Commission's Directorate-General for Research and Innovation (DG RTD), gave a brief overview of the support that the Commission provides through the Horizon 2020 (H2020) programme to research on COVID-19, and for alternatives to animal testing.

For COVID-19, the Commission allocated €780 million to more than 120 projects, he said. This funding is provided through the different mechanisms and instruments of H2020. In January 2020, through the first emergency call for Expression of Interest, 18 projects were funded (€48.2 million). This was followed by several other calls.

The fast-track call of the Innovative Medicines Initiative (IMI) in early March 2020 resulted in the funding of 8 projects (€72 million). The European Innovation Council (EIC) accelerator SME call later in March funded 36 projects (€165.6 million). The EDCTP (European and Developing Countries Clinical Trials Partnership) call for R&I in sub-Saharan Africa in April resulted in the funding of 20 projects (€25.2 million). In May, a 2nd Expression of Interest led to 23 projects (€128.2 million), while EIT HEALTH funded 15 projects (€6 million).

Altogether, these projects address various aspects of the pandemic, including epidemiology, public health, treatments, testing, prevention, cohorts to give insight into risk factors, the best clinical management, and optimal vaccination strategies, said Dr Desaintes. They cover many areas, from repurposing manufacturing to the socio-economic and psychological consequences of the pandemic.

Other H2020 COVID-19 research actions have been funded and pledged (€1 023 million). The Coalition for Epidemic Preparedness Innovations (CEPI) extended its portfolio of vaccines and global manufacturing capacity (€100 million); a new EU COVID-19 data sharing platform and adaptation of existing EU research infrastructures (€15.5 million); extensions of COVID-19-related projects, including clinical trials (€41.5 million + €17.9 million pledged).

Loans were given through the European Investment Bank (EIB) to the InnovFin Infection Disease Financial Facility (€178.5 million + €221.6 million pledged) to six companies, to expand manufacturing capacity for vaccines (Curevac and BioNTech), diagnostics (Scope Fluidics), and phase II clinical trials for treatment (Atriva, Immunic and AB Science). In addition, ICT Support was provided for the deployment of innovative robotics solutions in healthcare (€3.5 million pledged).

Of all these H2020 COVID-19 projects, only 10 projects (less than 8%) use animals, noted Dr Desaintes. These include 4 projects using non-human primates, and even in these projects the animal parts are often small. Therefore, we can assume that less than 0.2% of this huge funding effort goes to animal research.
In comparison, around 10% of these H2020 COVID-19 projects use alternatives to animal studies. He highlighted some examples.

One project generated 17 types of antibodies with the phage display technology from pre-pandemic healthy donors. These antibodies bind at the SARS-CoV-2 RBD-ACE2 interface. It will help provide opportunities to use antibodies in the treatment of COVID-19 patients.

Another project used supercomputers and AI to screen millions of molecules, looking at the reaction of each with the virus receptor (ACE2). By doing this with the first set of 440 000 molecules, the project identified Raloxifene (a treatment for osteoporosis) as a molecule that can block virus entry into cells. The Commission has provided an additional €1 million to test this molecule in clinical trials.

The Commission has been a strong supporter of the development of alternatives to animal testing over the past two decades, said Dr Desaintes, and has provided more than €800 million to more than 230 projects in this field.

H2020 is now officially over, though some projects are still starting in 2021. These include 7 new H2020 "alternative" projects relating to non-animal methods (total funding €84 million). Of these 7 projects, 3 are on animal-free safety assessment of chemicals (€60 million), involving complementary approaches. They will look at toxicity in the liver, lung, kidney and heart; developmental neuro-

toxicity and teratogenesis; motor deficit; non-genotoxic carcinogenesis; and endocrine disruption.

The other 4 projects will focus on next-generation Organ-on-Chip models (€24 million). One project will build a Heart-on-Chip for testing CVD drugs, another will study brain-gut axis to look for infectious diseases (including HIV), another concerns the cancer-lymph node for personalised treatment against metastasis, and the last is a synovial chip to study rheumatoid arthritis.

Dr Desaintes contacted researchers of 72 H2020 "alternative" projects, and around half of the project coordinators responded. Of these, 11 (35%) said they have redirected their activities to COVID-19 research, while 17 (55%) said their tools had contributed to COVID-19 research.

These projects included one developing microfluidic devices with stem cells derived from human vascular endothelial cells, which it adapted to model COVID-19 associated thrombus formation and also used immune cells to model SARS-CoV-2 induced inflammation. Another project, involving microphysiological systems that reproduce the minimal functional entity of the brain, used their system with coronavirus. Another project adopted their air–liquid interface cultures of nasal epithelial cells to study SARS-CoV-2 pathogenesis, as it corroborates the expression of ACE2 in goblet/secretory cells and ciliated cells.

A lot of organoids were used to contribute to COVID-19 research. For example, small intestinal organoids, where SARS-CoV-2 infected enterocytes produce infectious viral particles and trigger a viral response. Other projects used their liver, lung, cardiac, kidney and blood vessel organoids to study SARS-CoV-2.

Other alternative methods have been used to study COVID-19. Human primary lung cells have been used to study how SARS-CoV-2 infection results in fat build-up inside lung cells (the cholesterol-lowering drug fenofibrate is currently being tested). Other projects, for example, look at the bio-coating of SARS-CoV-2 spike protein for drug and siRNA screening; iPS differentiated into upper airway for infection studies; and Adverse Outcome Pathway framework for modelling the pathogenesis of COVID-19 (JRC's CIAO project). In conclusion, Dr Desaintes said that the Commission is a strong supporter of research into COVID-19, and also of alternatives to animal testing. Support to alternatives to animal testing will be continued during Horizon Europe (2021-2027), which was officially launched on 2 February 2021.

Bridging Across Methods in Biosciences

Maurice Whelan, head of the chemical safety and alternative methods unit of the European Commission's Joint Research Centre (JRC), said that his unit also incorporates the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM).

Photo: © Maurice Whelan

Maurice Whelan (JRC)

He started his presentation by telling a story about Nature, the famous science journal. A lot of its prestige comes from the fact that it is over 150 years old. It started as one journal, but today there are over 60 different Nature journals, each dealing with a particular scientific topic. This reflects the huge growth in scientific endeavour, but also the extent of specialisation and diversification that has happened.

In fact, we have come a long way since the publication of the very first paper in the very first journal, nearly 350 years ago, he said. The scientific community currently supports 30 000 different journals, publishing about 2 million papers each year.

This diversity is apparent too when we look at the methods used to replace animals in science: *in-vitro*, *in-silico* and *in-chemico* methods, 'omics, human-derived stem cells, big data and AI, organ-on-chip, functional imaging, and high-throughput screening. All look at various aspects of human biology and physiology in different ways, said Prof Whelan.

Although these methods have a lot to offer in their own right, it is only through their integration that full replacement of animals will be possible. That sounds doable in theory, he said, but is proving challenging in reality. In fact, there is a sort of Catch 22 situation. We need dedicated scientific communities to develop these methods, but their specialisation and autonomy poses an unfortunate barrier.

These days, the demarcation between scientific communities does not so much follow scientific disciplines (e.g. biology, chemistry, physics), but is more about

the methods used for research, he said. This is strongly reflected in the non-animal testing domain, where we frequently refer to in-vitro, *in-vivo* and *in-silico* as being separate communities, each with their own societies, journals and conferences. This realisation led to the 2019 JRC report on Bridging Across Methods in Biosciences (BeAMS), which freely available on the JRC website[35]. It was produced through a collaboration with Annamaria Carusi, a philosopher of science who brought valuable insights, noted Prof Whelan.

In this study, we also looked at how trends, hot topics, openness in science, reproducibility and other factors are helping, or possibly hindering, cross-disciplinarity, Prof Whelan explained. We analysed barriers at macro level (research systems) and micro level (everyday science practices), how knowledge is or isn't shared, and how we can put better systems in place to bridge across existing knowledge communities, and build new ones.

He then talked about how the report's recommendations are being put into practice. The first example, was the JRC's review of non-animal models for seven different disease areas of biomedical research (p. 38). We did this in a structured and consistent way, to be able to compare across the different disease areas to get a transversal view of how progress is being made overall, he said.

One example of reconciling different community perspectives concerns the use of the terms 'model' and 'method', he said. The biomedical research community talks more about the research model, for instance, while toxicologists typically refer to a method (that usually incorporates a model). These are seemingly minor differences, somewhat semantic, but they are in fact very important to understand to be able to share knowledge and experience across scientific divides.

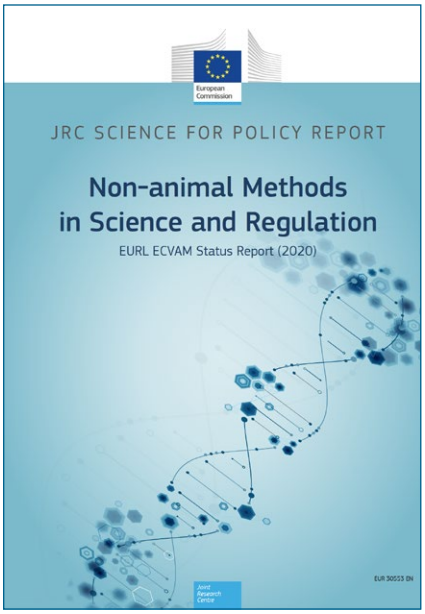
Another example presented was the Adverse Outcome Pathway (AOP) framework and how it represents a knowledge management system designed to bridge across the biosciences. This originated in the regulatory toxicology community and is a substantial programme at the OECD. It supports the application of non-animal approaches to chemical testing and assessment. The framework provides a bridge for connecting scientists working at different levels of biological organisation, whether at the molecular, cellular, organ, organism or whole-population level. For instance, AOPs can bring molecular toxicologists together with epidemiologists to efficiently pool knowledge and information for better risk assessment.
The JRC's CIAO[36] project, for example, brings together the mountains of multi-disciplinary knowledge emerging on COVID-19, to make sense of it all and to present it in a structured and transparent format. This provides an easily sharable and highly valuable resource for many interested communities.

Another bridging initiative is bringing together the rapidly-growing organ-on-chip community with the standards community. This initiative is looking at how standards can play a role in translating organ-on-chip approaches into impactful and widespread applications. For example, through the 'Organ-on-Chip: Putting Science into Standards' conference (28-29 April 2021) we are hoping to construct the right bridges at the outset to enable integration and foster co-creation.

One of my favourite examples of how to create a system to build bridges across method-centric domains is the In3 project[37], an innovative exchange programme for PhD students, said Prof Whelan. This EU H2020 Marie Skłodowska-Curie Action - Innovative Training Network brings young European scientists together from the *in-vitro* and *in-silico* worlds to promote an interdisciplinary approach to animal-free nanomaterial and chemical safety assessment.

And finally, he said, the best place to lay the foundations for more cross-disciplinary science is during formal education, at schools and universities. Courses need to be creatively constructed to develop a range of critical skills and to be more inclusive regarding content to broaden perspectives.

Prof Whelan concluded by saying that many of the things the JRC have been doing are summarised in the new EURL ECVAM Status Report (2020) on 'Non-animal Methods in Science and Regulation', freely available on the JRC's website[38].



35. https://ec.europa.eu/jrc/en/publication/eur-scientific-and-technical-research-reports/bridging-across-methods-biosciences
36. https://www.ciao-covid.net
37. https://estiv.org/in3/
38. https://publications.jrc.ec.europa.eu/repository/bitstream/JRC123531/jrc123531online.pdf

Moderated discussion

Wendy Jarrett *(Understanding Animal Research): Back in early 2020, global regulators agreed that animal studies would be required to show that candidate COVID-19 vaccines produced an immune response, before clinical trials using human volunteers would be allowed. So all the COVID-19 vaccines we have at the moment were tested in animals. Do the panel think that we could have developed these vaccines without any animal research or testing?*

Rhiannon David *(AstraZeneca): Though not my specific area, I can say from AstraZeneca's perspective that all the animal studies done were carefully considered and determined to be pivotal and highly relevant. They were also kept to a minimum, without compromising our robust safety assessment, and all done in rodents. We always look at what the critical animal studies are for the particular application.*

Dilyana Filipova *(European Coalition to End Animal Experiments): It is a common misconception that COVID-19 vaccine development followed the usual route, which is first animal testing and then human clinical trials. To get several vaccines approved so quickly, many of the animal studies were performed more quickly than usual, or were performed during or even after the first clinical trials with human volunteers.*

Regarding a question earlier about experiments with macaques, for mRNA vaccines (Pfizer and Moderna) these monkey studies were performed after hundreds, or even thousands, of people had already been vaccinated in phase I clinical trials. So, even if you read the literature, they say that mostly the results observed in human volunteers drive the decision about which tested vaccine candidates should proceed for further development. We also know that many of the animals used in experiments cannot even be naturally infected with the coronavirus, so this is a case where animal experiments were used but from a scientific point of view, these data were not decisive for the success of the vaccines.

Teri Schultz: *Is there enough funding for translating models described in scientific papers into standard operating procedures that can be followed by companies?*

Maurice Whelan *(JRC): In recent years, as illustrated by flagship programmes like EU-ToxRisk, research communities are doing a lot more towards standardisation, describing their methods in detail for a third party to reproduce them. The EURION Cluster of 8 projects is very mindful of developing their methods to be suitable for regulatory application. At EURL ECVAM, we are undertaking validation studies on 18 methods in 15 different facilities of EU-NETVAL, a network of highly qualified laboratories supporting us in validation activities across Member States.*

Christian Desaintes *(DG RTD): I would like to add that during the evolution of the programmes, things are becoming more integrated, with bigger projects bringing together all the various actors to cover all angles of R&I. We are funding three new H2020 projects on animal-free safety assessment of chemicals, for example, which form a cluster, integrating all actors in a multidisciplinary approach. We also have the European Innovation Council, which funds lots of translational aspects of research.*

Rhiannon David: *We work with different collaborators to evaluate these different methods, through internal budgets, studentships/post-doc funding, for adoption of these models. They are expensive and bringing them into more widespread use is going to require investment.*

Samantha Saunders *(PETA International Science Consortium): Can the panel comment on the opportunities for repurposing drugs (and even vaccine platforms) as a strategy for avoiding new animal tests?*

Rhiannon David: *Not specifically for re-purposing, but it is a good example where alternative models can be used. We need to drive confidence in these models and some of that is changing mind-sets, but ultimately it is about giving people confidence that the data we get from these systems is accurate and informative. With re-purposing drugs, we already have a wealth of animal data and some clinical, depending on the point we are looking at for re-purposing from. Therefore, there is an opportunity to use these models to demonstrate we can recapitulate those effects. So when you look at generating data for a new application we should have a lot more confidence in the predictions these models can give us in that context.*

Teri Schultz: *What can be done to speed up the process?*

Rhiannon David: *Regulatory acceptance is key. In my experience, regulators are open to using alternative models in regulatory submissions. We are starting to get to a point with organ-on-chip systems where we can start to submit those data. Those of us who are using them for generating safety data, should start submitting those data because that will be pivotal in driving change.*

Maurice Whelan: *I just wanted to add that the regulatory process varies for different sectors, so we need to appreciate that. We definitely need to understand that there was a time when toxicological data relied primarily on animal tests, and therefore it is understandable why information required by hazard and safety assessment processes are biased towards that type of data. Now that we have new non-animal data streams, it is right and proper we build a bridge from both sides. At JRC we have feet in both camps and we see a great willingness in the regulatory community to evolve and move forward, to reduce animal testing, and do a favour for the economy, so it is really now a case of how we do that together.*

Dilyana Filipova: *I wanted to add that regulatory requirements do not make scientific sense when there are much better*

models available now that do not involve animals. I think it is important to show regulators not only the validation and advantages of emerging methods, but that standard methods can have a low probability of success. If we had the same regulations decades ago we would not have some of the most common drugs used today, such as aspirin, because they are poisonous for most laboratory animals.

***Jarrod Bailey** (Center for Contemporary Sciences): Is the funding for NAMs sufficient, in the opinion of the panel? Many voices are calling it insufficient, especially relative to funding of animal-based approaches.*

***Christian Desaintes:** The budget going into animal-based research has declined. Funding is going more into translational aspects that are human-relevant.*

***Maurice Whelan:** Ultimately, we want to be doing more relevant research using the right tools. And what we are hearing is that there is a growing community believing that the right tools are non-animal tools, because they are more human-relevant.*

***Christian Desaintes:** The Commission is relatively open to freedom of research and this includes animal testing if it is needed, and also human-derived stem cells as long as it obeys certain ethical rules.*

***Laura Alvarez** (Cruelty Free International): How are we going to drive this much-needed transition to eliminate animal tests without setting clear targets, like the Paris Agreement for climate change? For example, the US EPA has set a deadline to phase-out animal studies by 2035. Will the EU do the same?*

***Rhiannon David:** In safety testing, we have the opportunity to be a bit more bold and innovative and accelerate the use of some non-animal models. Some of the new modalities of drugs we are developing now don't have any relevant animal models, so we need to look at the alternatives. What we are lacking from the animal models is the mechanistic understanding of drug toxicities and adverse events. I think these models give us that potential.*

***Dilyana Filipova:** I think it is very important to have a strategy and specific targets, because without deadlines and milestones it will be hard to define an agreed plan. This is something that should be discussed and evaluated with many different stakeholders, and a stepwise process developed with a clear purpose and goal to eliminate animal experiments and move towards more human-relevant methods.*

***Maurice Whelan:** I think it laudable what US EPA has done, but let's be clear that it is more of a political target that to my knowledge has no legal basis. In Europe we have Directive 2010/63/EU for the protection of animals used for scientific purposes, which already provides a legal basis to say that if we have a scientifically valid method available it must be used. The other contextual aspect to be aware of is that the EPA declaration is specific for the testing of certain types of chemicals, like pesticides or industrial chemicals; whereas the EU Directive covers the use of animals for all kinds of scientific purposes across any sector. It should also be kept in mind that half the animals are used for basic and applied research, and not in regulatory testing.*

We indeed have milestones and a strategy in Europe. We do not have policy-adopted hard deadlines in terms of banning anything, but we are very much strategic in our approach. I don't just mean the Commission in terms of strategic funding priorities, but also across industry. We have Cosmetics Europe, Cefic, ECETOC and many other industry organisations with elaborate science programmes that are very strategic. Then we have the European Partnership for Alternative Approaches to Animal Testing (EPAA), which brings the Commission and industry together. So in summary, we have always been committed to targets and milestones. But that is different from saying let's impose a blanket ban on animal testing by some arbitrary date - that is not the EU's approach.

***Christian Desaintes:** It is important to set a strategic target, but it is difficult to set a date. If you look back to 1950-1960, animal models were used as a kind of 'black box'. I think since then in biomedical research we have made enormous progress. New tools have allowed a move away from the 'black box'. For example, in the 1970s monoclonal antibodies were developed, in the 1980s PCR, the 2000s saw human genome sequencing and other 'omics, then iPSC, and now CRISPR-Cas9 enables us to edit genes. With all these tools, and the concurrent development of bio-informatics and AI-tools, I think we are getting closer to replacing animal testing. I think that in a way we need to trust in science, and not forget that all these major discoveries are very often game changers coming from unexpected directions.*

5 discussion points

- ***New approaches using human tissues/cells are becoming a natural choice in drug development and other areas, e.g. by being more human-relevant.***
- ***Both animal and non-animal approaches are used in COVID-19 research.***
- ***It is important to set strategic targets but difficult to set dates/milestones.***
- ***Regulatory acceptance of alternative methods is key to their wider adoption.***
- ***Giving people confidence that the data generated by non-animal models is accurate and informative will ultimately drive confidence in them.***

Closing remarks

Photo: © Maurice Whelan

Maurice Whelan
(JRC)

Q: Teri Schultz (co-moderator): I am surprised that at this conference people from so many parts of the puzzle are on the same page. Replacing animals is something everybody wants to do.

A: Maurice Whelan: It has been a wonderful two days. The sheer amount of participants, I think over 1 000 at one point, has surpassed our expectations. The diversity of participation shows too how the Three Rs really is a cross-cutting issue in our society. There has definitely been a lot of consensus, and obviously some healthy debate about how we want to move forward, but there's definitely a unified sense about the direction of travel.

Q: But no obligations to move towards that, unless you are driven by ethics?

A: I would not agree with you there. We have a strong legal basis in the EU. The protection of animals is laid down in the Lisbon Treaty and then reflected in our Directive that is transposed into national legislation. The rules are very simple from a legal perspective - if there is a scientifically valid alternative it has to be used irrespective whether it's in research or regulatory testing. Of course, that raises the question of what is 'scientifically valid'. I think that is what we were discussing a lot today in terms of what fit-for-purpose means and who decides. But irrespective of that, the legal basis is there.

Q: People talked about changing mind-set. How much is that your responsibility, making sure everyone has the evidence for potentially changing minds?

A: For me, the programme of this conference really worked, and it reflects what you are touching on there. Changing mind-sets is not just about cutting-edge science. It's just as important to focus on transparency and knowledge-sharing, on education and training, and discussions about building confidence and gaining trust. This really reflects the multifaceted nature of the topic, and the need to move ahead on many fronts together, and the progress we can make when we do.

Q: Is EU regulation always the best method to impart ways to build trust, or do you also need less top-down methods, talking to consumers and everyone involved in the product chain. Who would you like to see step up and help more than they are now?

A: You know what they say, if you want to go fast go alone, but if you want to go far, then go together. And, our EU policy wants us to go very far, to achieve full replacement of animals used in science. For that we need a concerted, sustained, collective effort, involving many diverse communities, both scientific and non-scientific, and that came through loud and clear during this conference.

We need to build bridges. Working at the JRC, I have the privilege of engaging with so many different communities who have a lot of skin in this game and I know each and every one of those communities, whether industry, NGOs, government organisations, regulatory communities or academics, all want the same thing.

Q: Do national government's need to play a stronger role, at very least in funding?

A: I believe there is a lot of substantial funding at Member State level. But nowadays it's getting harder to identify and label activities as specifically funding alternatives. On the one hand, we have really wanted dedicated budgets for alternatives, but on other hand, in recent years, we want to mainstream non-animal methods to a point where they are so common they needn't actually be called alternatives any more.

Q: People were asking about the timeline. Are you satisfied with the progress and can we really get there? Doctors and vets say you cannot completely replace animal testing. At the same time, experts say animal testing is sufficient for testing products and in other sectors. Is it possible?

A: The first part is whether it is happening fast enough. Although I definitely see momentum and willingness, in my own personal opinion however, I am not satisfied with the pace.

The second part of the question is really important. What is missing in our non-animal science goal? One thing I feel is missing is belief. There seems to be lots of hope and enthusiasm out there, and excitement, but I still don't think there is enough collective belief that full replacement is really possible. If you think of this as a moon shot there can't be any half measures, we need to push the envelope. Either you are going to the moon or you're not. We can't be content with only getting half-way there. In my view therefore, the most important ingredient for success is belief. So we need more people to truly believe that this is possible.

NOTES

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