

Better ways to do research:

An overview of methods
and technologies that
can replace animals in
biomedical research
and testing.



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Humane Research Australia is a not-for-profit organisation that challenges the use of animal experiments and promotes the use of more humane and scientifically valid non-animal methods of research.

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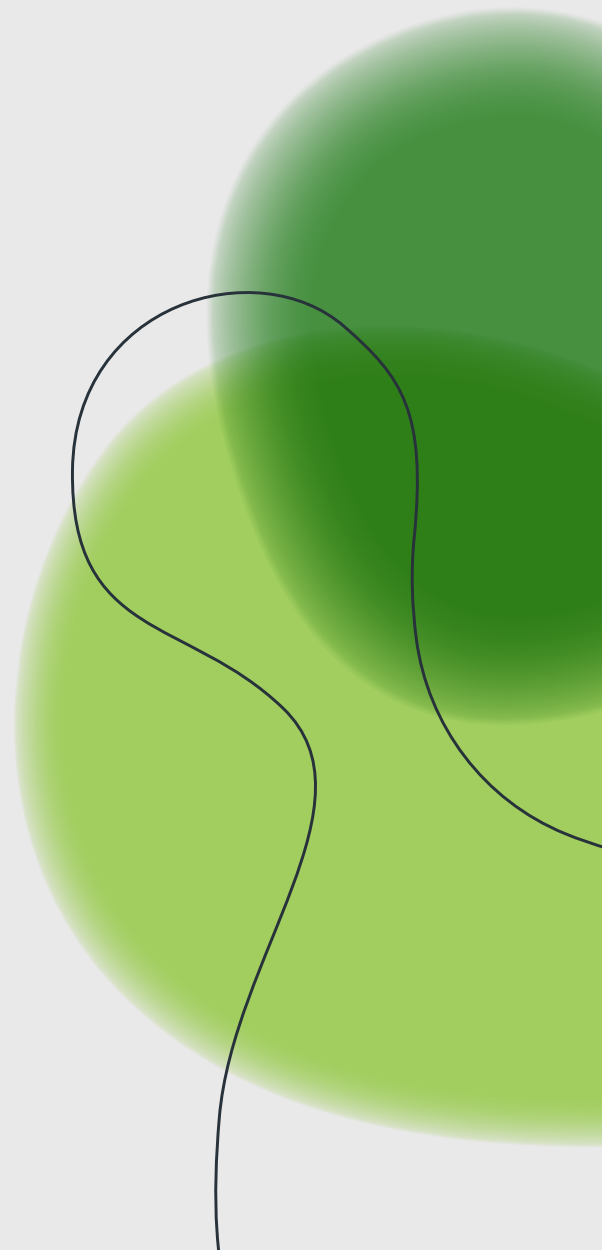
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Better ways to do research:

**An overview of methods and technologies
that can replace animals in biomedical
research and testing**

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May 2019



Foreword

Why I wrote this overview

“But isn’t animal research a necessary evil?” This is a common response when I mention that I am the president of Humane Research Australia. For several years now, I have wished for a publication I could recommend to people who are genuinely interested in alternatives¹ to animal research², but I have found scientific research articles hard to locate and difficult to read. Summaries of alternative methods are available, but they are usually limited to a few pages. This overview in plain English is intended to fill this gap.

My sources are mostly scientific publications in peer-reviewed journals and government reports, and I provide references so that the interested reader may find out more from the original article or report³. I have summarised what I found in the scientific literature and use many quotations because I think researchers who have done the work can often explain it best.

The focus here is on animal research conducted mainly for human medicine or other human “benefit”, which is often questionable. Animals are also used for studies in veterinary medicine.

This overview of alternatives to animal research and testing, as well as the use of live animals in education and training, was written in late 2018 and early 2019. In this rapidly growing area of research, existing methods and technologies are being constantly improved and new ones added. It is also a very complex area that is difficult to access for a layperson. I have done my best to give a sense of the available information at the present and hope that readers may find it informative and useful.

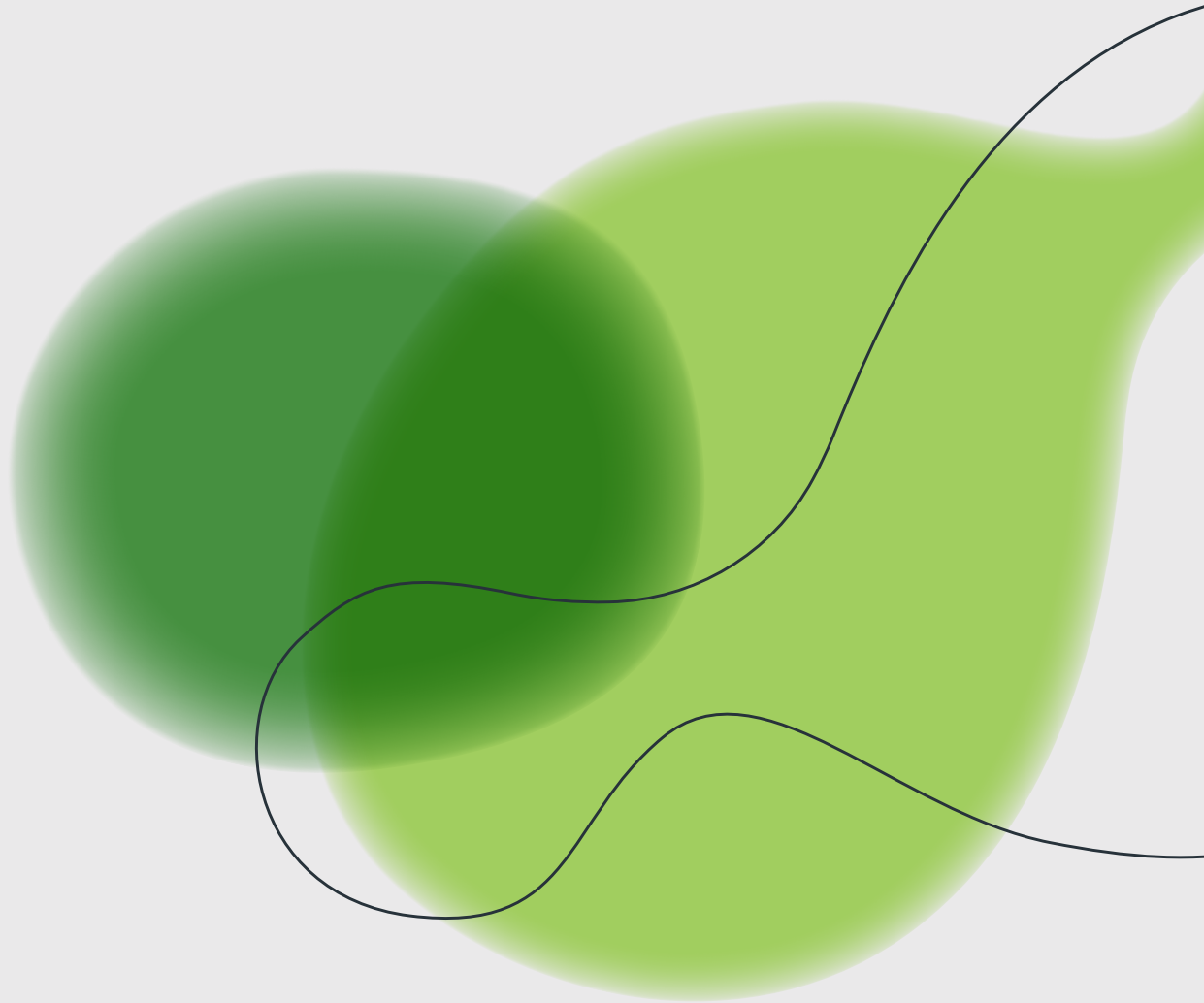
¹ The term “alternative” is used as relating to methods that depart from or challenge traditional norms. It is not used as offering another, equally valid possibility or choice.

² I use the terms animal research and animal experimentation interchangeably with animal referring to non-human animals.

³ While some articles are behind paywalls, authors are usually willing to provide a copy of their publication when contacted via email. A contact email address is usually included in the article abstract, which is available for free.

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Summary

Better ways to do research: An overview of methods and technologies that can replace animals in biomedical research and testing.

Each year, millions of non-human animals⁴ worldwide are harmed by animal experimentation. It has been estimated that more than 115 million animals are used per year to supply the biomedical industry⁵. The countries that use the most animals include China, the US, Japan and Australia. Broadly, the types of research that use animals consist of a) fundamental research (also called basic research), b) applied (or human disease) research, and c) testing (or regulatory testing).

There are alternatives to using animals. New – and not so new – methods and technologies that can replace live animals in research, testing, education and training include:

1. In-vitro methods (performed with microorganisms, tissues, whole cells or parts of cells in test tubes, Petri dishes etc.)
2. In-silico (computer-based) methods
3. Studies with human volunteers
4. Simulators

1. In-vitro (test tube) methods

3D tissues and microfluidic devices: Organoids, organs-on-chips

Organoids are a miniature and simplified version of a (human) organ. Organoids are grown in-vitro in three dimensions. They allow researchers to study disease and treatments in the laboratory. Mini organs can also be grown on microchips. Researchers have used microchip manufacturing methods to engineer microfluidic⁶ culture devices that can mimic the structures and functions of living human organs.

Organoids, organs-on-chips – Useful for/ can replace animals in:						
Chemicals/ drug testing for toxicity & effectiveness	Regenerative medicine & other treatments	Personalised/ precision medicine	Organ/ disease studies	Large scale & fast studies/ tests	Education & training	Provides access to human tissues
✓	✓	✓	✓	✓		

⁴ hereinafter referred to as animals

⁵ Akhtar, 2015

⁶ At the micro scale, often flowing through channels.

Biobanking

To study human cells and tissues, researchers need a readily available supply of these human biological samples. They are stored in so-called biobanks or tissue banks. Biobanks use tissue that is left over from clinical procedures such as surgery, from dead bodies, or they collect tissue specifically for research. They also store organoids.

Biobanking – Useful for/ can replace animals in:						
Chemicals/ drug testing for toxicity & effectiveness	Regenerative medicine & other treatments	Personalised/ precision medicine	Organ/ disease studies	Large scale & fast studies/ tests	Education & training	Provides access to human tissues
						✓

Omics technologies

The term “omics technologies” refers to areas of study in biology whose names end in “omics”, such as genomics (the study of the genome of an organism). The science of “omics” reflects diverse technologies with a focus on studies of life processes, such as comprehensive studies of genes, proteins and metabolites of an organism.

Omics technologies – Useful for/ can replace animals in:						
Chemicals/ drug testing for toxicity & effectiveness	Regenerative medicine & other treatments	Personalised/ precision medicine	Organ/ disease studies	Large scale & fast studies/ tests	Education & training	Provides access to human tissues
		✓	✓	✓		

Stem cell technologies

Stem cells are unspecialised or undifferentiated cells with the ability to self-renew, and to differentiate to produce specialised cell types in the body.⁷

Stem cells – Useful for/ can replace animals in:						
Chemicals/ drug testing for toxicity & effectiveness	Regenerative medicine & other treatments	Personalised/ precision medicine	Organ/ disease studies	Large scale & fast studies/ tests	Education & training	Provides access to human tissues
✓	✓	✓	✓			

⁷ Stem Cells Australia, 2018

3D and 4D bioprinting

Bioprinting involves the precise layering of cells, biologic scaffolds, and growth factors with the goal of creating bioidentical tissue for a variety of uses.⁸ 4D bioprinting aims to create dynamic 3D patterned biological structures that can transform their shapes or behaviour under various stimuli. For example, 4D bioprinted materials are capable of changing their shape over time.⁹

3D and 4D bioprinting – Useful for/ can replace animals in:

Chemicals/ drug testing for toxicity & effectiveness	Regenerative medicine & other treatments	Personalised/ precision medicine	Organ/ disease studies	Large scale & fast studies/ tests	Education & training	Provides access to human tissues
✓	✓		✓	✓		

Robotic testing

Researchers, in particular those in the pharmaceutical industry, have developed automated methods to test biological activities of thousands of chemicals that used to be tested in animals. This is called high-throughput testing or robotic testing.

Robotic testing – Useful for/ can replace animals in:

Chemicals/ drug testing for toxicity & effectiveness	Regenerative medicine & other treatments	Personalised/ precision medicine	Organ/ disease studies	Large scale & fast studies/ tests	Education & training	Provides access to human tissues
✓			✓	✓		

⁸ Bishop, et al., 2017

⁹ Li, Zhang, Akpek, Shin, & Khademhosseini, 2017



2. In-silico (computer-based) methods

Prediction methods and tools

A range of in-silico prediction methods and tools for the evaluation of toxicity have been developed, such as structure-activity relationships (SARs) and quantitative structure-activity relationships (QSARs), read-across, physiologically-based pharmacokinetic (PBPK) models, expert systems, read-across structure activity relationships (RASAR), OECD QSAR Toolbox, REACHacross, Toxtree, and Toxmatch.

Prediction methods and tools – Useful for/ can replace animals in:

Chemicals/ drug testing for toxicity & effectiveness	Regenerative medicine & other treatments	Personalised/ precision medicine	Organ/ disease studies	Large scale & fast studies/ tests	Education & training	Provides access to human tissues
✓			✓	✓		

Other in-silico approaches

Adverse Outcome Pathways (AOPs) provide the biological explanation for a single toxic event.¹⁰ Integrated Approaches for Testing and Assessment (IATA) are approaches for making decisions about the toxicity of substances that are based on multiple information sources.

AOPs and IATA – Useful for/ can replace animals in:

Chemicals/ drug testing for toxicity & effectiveness	Regenerative medicine & other treatments	Personalised/ precision medicine	Organ/ disease studies	Large scale & fast studies/ tests	Education & training	Provides access to human tissues
✓			✓	✓		

Computer modelling

A computer-based model or simulation is a computer program that is designed to simulate a physical or biological system or situation. Computer models can link many processes together, something which is not possible to achieve with animal models.

Computer modelling – Useful for/ can replace animals in:

Chemicals/ drug testing for toxicity & effectiveness	Regenerative medicine & other treatments	Personalised/ precision medicine	Organ/ disease studies	Large scale & fast studies/ tests	Education & training	Provides access to human tissues
✓		✓	✓		✓	

¹⁰ Taylor, 2019

3. Studies with human volunteers

Post-mortem studies

Donated tissues after the death of a person can be studied to gain insight into cell-level changes in human illnesses, and cadavers can be used in training surgical skills. For example, post-mortem brain studies are useful to gain more knowledge about psychiatric illnesses, in particular in combination with the approaches of genomics and proteomics (the study of the structural and functional aspects of total proteins of an organism or system using high-throughput technologies).¹¹

Post-mortem studies – Useful for/ can replace animals in:

Chemicals/ drug testing for toxicity & effectiveness	Regenerative medicine & other treatments	Personalised/ precision medicine	Organ/ disease studies	Large scale & fast studies/ tests	Education & training	Provides access to human tissues
			✓		✓	✓

Population-based studies

Epidemiology is the study of diseases and other health-related states in groups (populations) of people, in particular how, when and where they occur. Epidemiologists want to discover what factors are associated with diseases (risk factors), and what factors may protect people against disease (protective factors).

Population-based studies – Useful for/ can replace animals in:

Chemicals/ drug testing for toxicity & effectiveness	Regenerative medicine & other treatments	Personalised/ precision medicine	Organ/ disease studies	Large scale & fast studies/ tests	Education & training	Provides access to human tissues
✓			✓			

Microdosing

Microdosing involves the administration of very low doses of a substance (sub-therapeutic). When testing a new compound or drug, microdosing can provide useful information to help decide whether the new compound or drug should be developed further, and whether it may be safe to progress to further human testing.

Microdosing – Useful for/ can replace animals in:

Chemicals/ drug testing for toxicity & effectiveness	Regenerative medicine & other treatments	Personalised/ precision medicine	Organ/ disease studies	Large scale & fast studies/ tests	Education & training	Provides access to human tissues
✓						

¹¹ Yadav, Tanveer, Malviya, & Yadav, 2017



4. Simulators

Simulators are either virtual reality (VR)-based or physical model (PM)-based. Apart from replacing live animals in education and training, VR simulators have great potential for training people in remote locations, for example, training students and surgeons in developing countries.

Simulators – Useful for/ can replace animals in:						
Chemicals/ drug testing for toxicity & effectiveness	Regenerative medicine & other treatments	Personalised/ precision medicine	Organ/ disease studies	Large scale & fast studies/ tests	Education & training	Provides access to human tissues
		✓	✓		✓	



Collaborative efforts to replace animal experimentation

Governments, the scientific community, industry and other stakeholders, in particular in the EU and the US, have started to make efforts to pool knowledge and resources to replace animal experimentation with more humane, more human-relevant, and often cheaper and faster methods. This includes, for example, the development of policies and tools, working together to build large databases, developing plans for the further development of particular technologies (such as organs-on-chips), and collaborating on the validation of new methods and technologies.



Why it matters

For a long time, the use of animals in research, testing, training and education has been considered a necessary evil. More and more, people question the ethics of this approach. At the same time, the animal research community increasingly recognises the problems with animal research: it is costly, lengthy and not very effective. Also, it may have held back the discovery of treatments and cures for humans because they did not work well in animals.

The main alternatives to the use of animals in the laboratory are new in-silico and in-vitro approaches. Studies with human volunteers and simulators also play an important role. Some of these methods are used in combination for greater effectiveness. So far, most progress in the development of alternatives has been made in the area of toxicology. The new methods and technologies are not yet perfect, and some of the current methods that are deemed to be alternatives might still use animal parts.

Animal researchers argue that the new methods can't replace all areas of current animal research. Considering the ethics of using animals for the purported benefit of humans and the many shortcomings of animal research, this is a compelling argument for speeding up the development of human-relevant research and testing without animals.

We need urgent change. From an animal rights perspective, it was never okay to inflict pain and suffering on animals for the real or perceived benefit of humans. For proponents of animal welfare, the use of animals is justified as long as harm is minimised. With awareness of the many shortcomings of animal research and testing and increasing availability of better ways, animal research is no longer justified.

With greater investment in innovative and promising non-animal methods, firm policy initiatives and robust collaborations of all interested parties, better treatments and cures for human diseases can be developed. This will also end the suffering of millions of animals.

Introduction

This is an overview of methods and technologies that can replace live animals in research, testing, education and training. It is divided into four sections:

- In-vitro methods (performed with microorganisms, tissues, whole cells or parts of cells in test tubes and Petri dishes)
- In-silico (computer-based) methods
- Studies with human volunteers
- Simulators

This is followed by a section on efforts by governments and the scientific community to replace animal experimentation, as well as concluding reflections on why the availability and further development of alternatives matter. A glossary is also included.

But first of all, I provide a few general observations and facts about animal research.



What types of research use animals?

The types of research that use animals are usually called fundamental or basic research, applied or human-disease research and testing (regulatory testing). Basic research is curiosity driven and, unlike applied research, it is not necessarily designed to answer specific questions or solve practical problems. It is exploratory and aims to increase and advance scientific knowledge. Applied research aims to solve specific practical problems, such as using animals as a model to seek a cure for a human disease or condition. Animals are also used in education and training – from preschool to postgraduate level and in professional development. There is no legal requirement for animal experimentation in basic and applied research, nor for it to be part of

education and training. Individual scientists decide what is worth studying and whether or not they will use animals.

The situation is different for the third type of research, regulatory testing. Government regulators require that new consumer products, medicines, and industrial and agricultural chemicals are tested to identify potential dangers to human and animal health, as well as to the environment. For some product types (drugs and vaccines, biologicals¹²), animal testing includes testing for efficacy as well as safety (toxicity). Current laws in many countries make it difficult to avoid using animals for regulatory testing, although with the development of new methods and technologies this has started to change.

Available statistics differ in how they describe and count procedures that use animals. They don't usually explain the purposes of animal research by these three categories. The descriptions provided by different countries also vary, as does the use of animals by category. But generally, basic research accounts for roughly half of all animals used in research. The three figures (below) are examples of statistical reporting on the use of animals for scientific purposes. These are the most recent statistics available from the European Union (EU), the United States (US) and Australia.

¹² Biologicals, or biologics, are drugs that are made from living organisms or contain components of living organisms.

Purposes of experiments, European Union 2011

2%

Education & training

2%

Diagnosis of disease

9%

Toxicological & other
safety evaluation

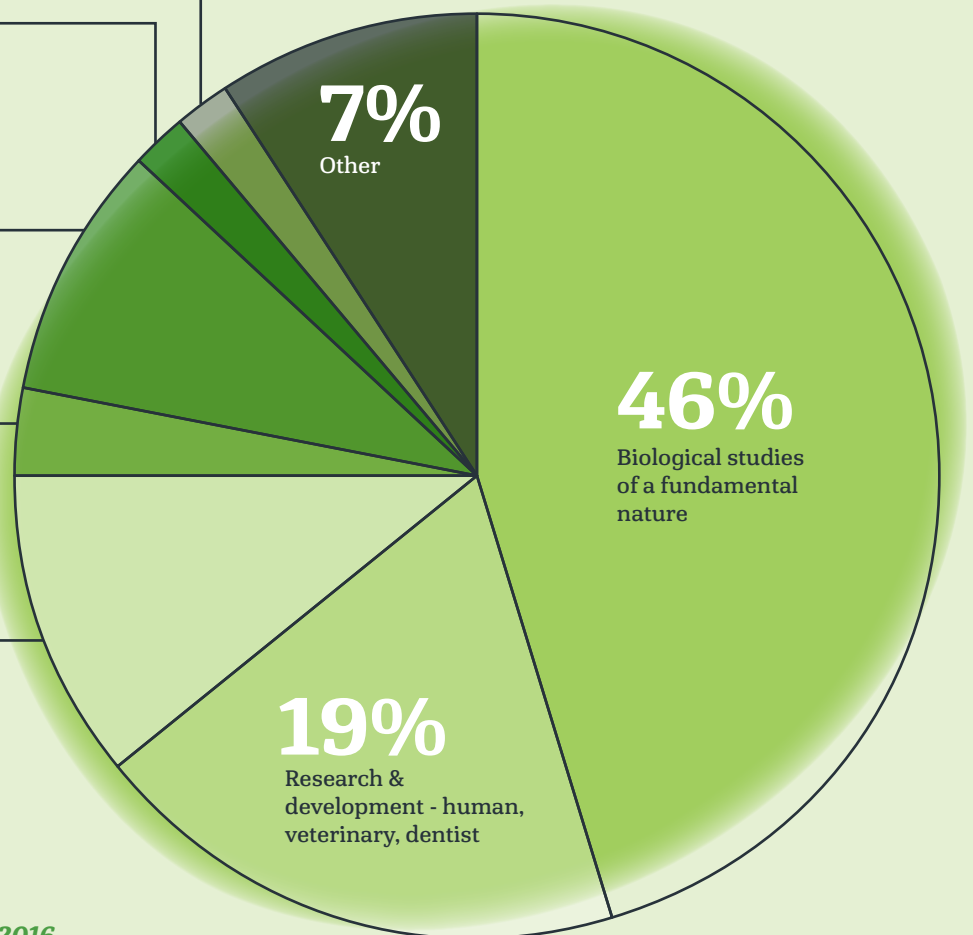
3%

Production and quality
control of products for
veterinary medicine

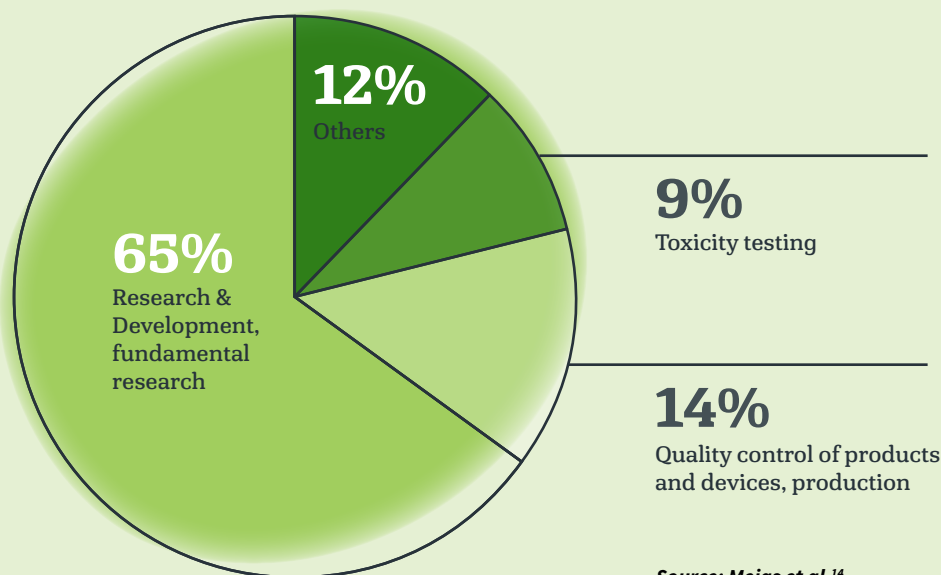
11%

Production & quality control
for products for human
medicine and dentistry

Source: European Commission¹³



Purposes of experiments, US 2016



Source: Meigs et al.¹⁴

¹³ European Commission, 2013, p. 6

¹⁴ Meigs, Smirnova, Rovida, Leist, & Hartung, 2018

Purposes of experiments, Australia 2016

0.27%

Stock Maintenance

0.30%

Regulatory product testing

2%

Achievement of educational objective

1%

Production of biological products

4%

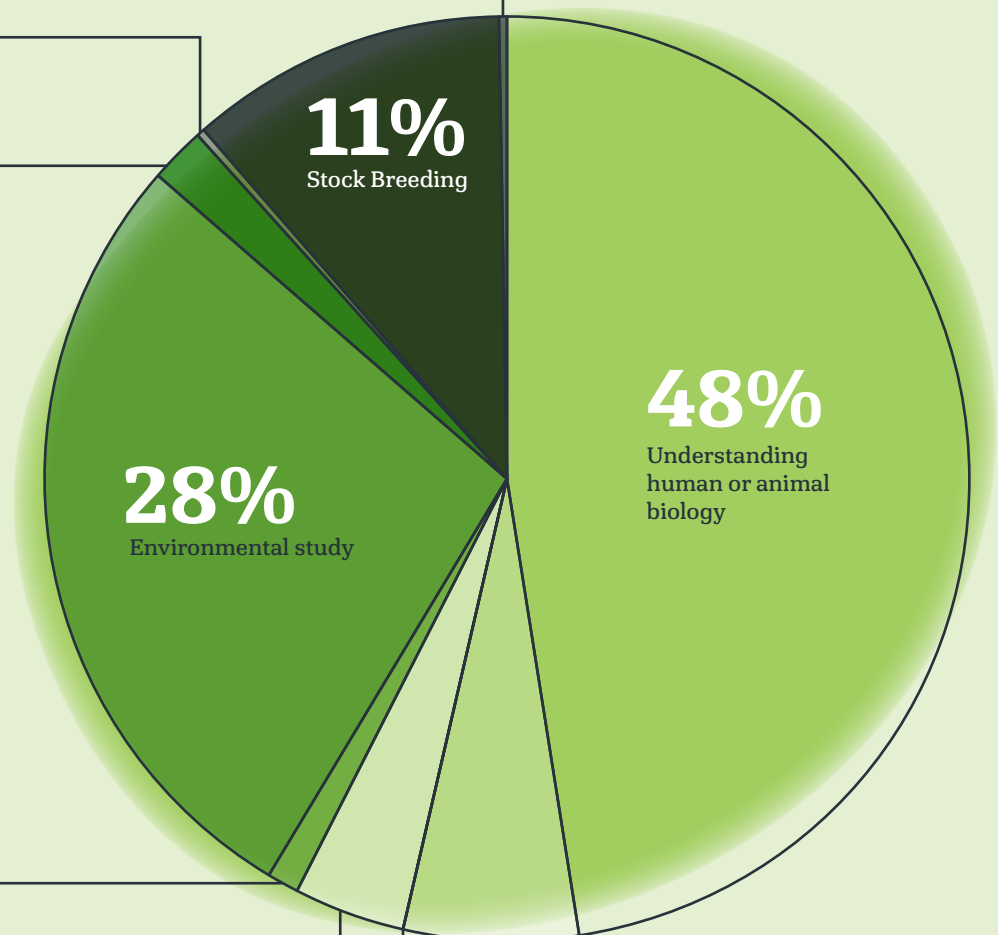
Improvement of animal management or production

0.02%

Diagnostic procedures

6%

Maintenance and improvement of human or animal health and welfare



Source: Humane Research Australia¹⁵

Mice and rats are the most commonly used animals in research. In 2011, rodents and rabbits represented 80% of all animals used in research in the EU. The second most-used group were cold-blooded animals, such as reptiles, amphibians and fish (12.4%) while birds accounted for 5.9%.¹⁶ However, in recent years the use of zebrafish has soared, partly due to their lower cost compared to mammals. In the UK, they are now the second most-used animals after mice.¹⁷

¹⁵ Humane Research Australia, 2016

¹⁶ European Commission, 2013

¹⁷ Home Office, 2015

How many animals are used?

We do not know how many animals are used worldwide for research, testing and teaching purposes. Not all countries release statistics about animal use. In Australia, for instance, only four states provide statistics while in the US, the numbers of mice, rats, fish and birds used are not known because these animals are not counted. From published data and estimates we can ascertain that:

- In Australia, around 9 million animals were used in 2016¹⁸
- In the EU's 28 member states, just under 11.5 million animals were used in 2011¹⁹
- In the US, an estimated 26 million animals were used in 2010, with 96% of these being mice, rats, fish and birds²⁰
- In Canada, in 2016, 4.3 million animals were used in research, teaching, and testing. The majority of animals (57.3%) were used in basic research²¹
- In South Korea, 3 million animals were used in 2017²²
- An estimate of worldwide use suggested a figure of 115.3 million animals used for research in 2005²³

From what statistics are available, it appears that, overall, the number of animals used in research has been stable in recent years²⁴ although in some countries, it has increased. In particular, the number of genetically-altered (transgenic) mice has risen, especially in basic research.²⁵



**Around
9 million**
Animals used in
Australia (2016)



**Just under
11.5 million**
Animals used in the
28 EU countries (2011)



**An estimated
115.3 million**
Animals used
worldwide (2005)



The number of animals
used in the laboratory
has been constant
over recent years

¹⁸ Humane Research Australia, 2016

¹⁹ European Commission, 2013

²⁰ The Hastings Center, no date

²¹ Meigs, et al., 2018

²² Meigs, et al., 2018

²³ Taylor, Gordon, Langley, & Higgins, 2008

²⁴ Meigs, et al., 2018

²⁵ Daneshian, Busquet, Hartung, & Leist, 2015; Timoshanko, Marston, & Lidbury, 2017



What animal welfare measures are meant to protect animals?

In animal experimentation, researchers are permitted to use procedures that would be illegal outside of the laboratory, such as artificially producing spinal cord injury in rabbits.²⁶ However, there are laws, regulations and codes of practice that direct researchers to limit harm to the animals they use. The most widely accepted guidance on limiting harms to animals in biomedical research comprises the principle of the 3Rs: Replacement, Reduction and Refinement. The 3Rs were proposed by William Russell and Rex Burch in the late 1950s and are today embedded in many laws, regulations and codes governing animal use.²⁷ It was summarised in a 2015 article as:

“In essence, they allow animals to be used in scientific research only when they cannot be replaced with non-animal alternatives, when the number of animals has been reduced as much as possible given the research goals, and when procedures and housing have been refined to minimize welfare impacts”.²⁸

Animal experimentation regulations vary around the world. Many countries require an institutional or project license before research using animals can be carried out. There is also usually a requirement for a group made up of representatives of veterinarians, scientists, animal welfare organisations and the general public to oversee the ethical conduct of individual projects. In Australia, these are called Animal Ethics Committees (AEC) and in the EU, all states have a national committee for the protection of animals used for scientific purposes. In Australia, the members of AECs are usually appointed by the institution that carries out the animal experiments. The public does not have easy access to their minutes and reports. It is generally not possible for a member of the public then, to assess whether the use of animals in a particular project could have been avoided because alternatives were available. In other words, the transparency of the process that determines decisions on the use of animals is questionable.²⁹



The 3Rs – Replacement, Reduction, Refinement

– are meant to protect animals in research testing.



The public does not have enough information to judge whether or how the 3Rs protect animals.

²⁶ Leonard, Thornton, & Vink, 2014

²⁷ Franco, 2013

²⁸ Franco, 2013, p. 2

²⁹ Merkes & Buttrose, 2019

Why do we need alternatives to animal research?

The use of animals in research – and the moral status of animals more generally – has been and remains surrounded by ethical controversy. Answers to the question “do we have the moral right to use animals for our purposes?” have been approached from many angles. Broadly, there are two positions: animal welfare and animal rights. Animal welfare is concerned with minimising suffering, while the animal rights position considers the use of animals as our resources to be morally wrong.

Animal liberationists, for instance, call for equal consideration of interests. They question the right of humans to assume that our interests must always prevail. For example, Peter Singer noted 30 years ago:

“And the basic right that animals should have is the right to equal consideration. This sounds like a difficult idea, but essentially it means that if an animal feels pain, the pain matters as much as it does when a human feels pain - if that pains hurt just as much. Pain is pain, whatever the species of being that experiences it”.³⁰

On the other hand, supporters of the animal welfare position find it morally acceptable to use animals in research as long as their wellbeing is considered. The 3Rs represent an animal welfare position, particularly in the requirements for refinement and reduction.

Recent surveys indicate a growing concern with the suffering of animals used in research, and the public is increasingly questioning whether animals ought to be considered as means to an end or as sentient beings with inherent value. In 2018, for instance, the Pew Research Center reported that 52% of Americans are opposed to using animals in scientific research.³¹

Ethics aside, there are other reasons why the use of animals in research and testing for human purposes should be replaced with better ways to do research.

In-vivo³² and traditional in-vitro methods (also called test tube experiments) are not good at predicting therapeutic outcomes and possible side effects during clinical trials with humans. As many as 95% of drugs that appear safe and effective in animals fail in humans.³³ Drugs for Alzheimer’s disease, for instance, have a 99.6% failure rate³⁴ while those for cancer³⁵ and heart disease also have a particularly high failure rate. A group of Canadian researchers made the following observation about the safety and effectiveness of specific drugs:

“Cardiovascular toxicity claims the highest incidence and severity of adverse drug reactions in late-stage clinical development. For example, Vioxx (Rofecoxib), originally designed to treat pain related to osteoarthritis and approved by the Food and Drug Administration (FDA) in 1999, was linked to over 27,000 cardiovascular-related deaths and myocardial infarctions (MI). It was withdrawn from the market in 2004, although later relicensed for more specific indications, with implementation of regulatory and transparency safeguards. In preliminary clinical investigations, the drug showed effectiveness in its target treatment and adverse events were not significant. It was not until four years of long-term clinical studies that it became evident that the risk of heart attack and stroke was actually two-fold higher with Vioxx compared to the control group. Some other compounds, such as Micturin (Terodiline, for urinary incontinence), Fen-phen (Fenfluramine/ phentermine, anti-obesity treatment), Seldane (Terfenadine, allergy medication), Zelnorm (Tegaserod, for irritable bowel syndrome), Meridia (Sibutramine, appetite suppressant), and Darvon/ Darvocet (Propoxyphene, analgesic drug), have all had a similar record in terms of adverse cardiovascular effects”.³⁶

³⁰ Singer, 1989, p. 1

³¹ Strauss, 2018

³² In vivo means “in the living”. In vivo methods are methods using a living organism/animal.

³³ e. g., Cummings, Morstorf, & Zhong, 2014; Marshall, Austin, Casey, Fitzpatrick, & Willett, 2018; Pound & Ritskes-Hoitinga, 2018; Seok, et al., 2013; Thomas, et al., 2016; Triunfol, Rehen, Simian, & Seidle, 2018; van der Worp, et al., 2010

³⁴ Cummings, et al., 2014

³⁵ Hutchinson & Kirk, 2011

³⁶ Savoji, et al., 2018, p. 1

How can these adverse drug reactions be explained, given that the drugs had to undergo safety (toxicity) testing? Thomas Hartung, Director of the Center for Alternatives to Animal Testing (CAAT) at Johns Hopkins University in the US offered the following explanation:

“Toxicology and effective risk assessment depend on scientific and technological information and should constantly adapt to these advances in this information. However, toxicology still largely relies on traditional assessment methods that were established decades ago and that have changed little despite scientific and technological progress. Consequently, safety assessments are often based on tests of unknown relevance and reliability and whose predictive validity has never been assessed objectively”.³⁷

The extensive use of animals in basic research raises another issue. Basic research does not aim to result in practical outcomes and it often doesn't. For example, a group of researchers examined articles in six highly cited basic science journals over a five-year period. They found that fewer than 10% of highly promising basic science discoveries enter routine clinical use within 20 years.³⁸ From a utilitarian perspective, which is often used by animal researchers, it can be argued that the many animal lives lost in basic research do not justify the benefits.

Opportunity cost, the loss of other options when one option is chosen, presents an additional problem. Given that animal research and testing has a high failure rate, we don't know to what extent new drugs that would be beneficial to humans have been overlooked, because they were harmful to animals.

In addition to producing misleading results, animal tests are also costly and lengthy.³⁹ The cost of drug development has been increasing and the number of new drugs approved every year has been decreasing over the last two decades.⁴⁰ The drug development process takes around 10 years and has been estimated to cost up to US \$2.5 billion.⁴¹ Economic considerations are in favour of more human-relevant, cheaper and faster methods.

“Unlike crude, archaic animal tests, non-animal methods usually take less time to complete, cost only a fraction of what the animal experiments that they replace cost, and are not plagued with species differences that make extrapolation difficult or impossible”.⁴²



Animals are not good models for predicting human clinical outcomes because of differences in physiology, metabolism and other differences between human and non-human animals.

“For decades, laboratory biologists have regarded animal models as a necessary evil. While some activists decry their use on moral grounds, even the most practical-minded researchers acknowledge fundamental problems with them. Animals are expensive, provide only imperfect replicas of human biology, and introduce numerous variables into experiments that can be difficult or impossible to control. These flaws aren't purely academic. Pharmaceutical researchers have struggled for years with late-stage development failures, in which drugs that look promising in multiple animal systems turn out to be useless or even toxic in humans. Nonhuman models have simply been the least bad tool for detailed studies on human biology”.⁴³

³⁷ Hartung, 2009a, p. 93

³⁸ Contopoulos-Ioannidis, Ntzani, & Ioannidis, 2003

³⁹ Meigs, et al., 2018

⁴⁰ Zhang, Korolj, Lai, & Radisic, 2018

⁴¹ Ahadian, et al., 2018

⁴² Ranganatha & J, 2012, p. 32

⁴³ Dove, 2018

In-vitro methods

In-vitro methods (the Latin phrase means “in the glass”) are also commonly known as test-tube methods although this kind of research is traditionally done in flasks or Petri dishes as well as test tubes. In-vitro tests and experiments are largely performed outside living organisms and involve tissues, microorganisms, cells or other small parts of biological material.

This section provides an overview of recently developed in-vitro methods that can replace experiments or tests with live animals. They include 3D tissues and microfluidic devices, such as organoids (mini versions of organs) and organs-on-chips, biobanking, omics technologies, stem-cell technologies, 3D and 4D bioprinting, and robotic testing.

3D tissues and microfluidic devices

For almost a century, scientists have grown cells, such as bacteria or human tissue, in Petri dishes. A Petri dish is a shallow glass or plastic dish with a lid that is used by scientists for microbiological studies. It contains a culture that feeds the cells so they can grow. The cells rest at the bottom of the dish and spread out as they multiply in a two-dimensional (2D) direction. Since this is not how organs grow in a living body, scientists have developed ways in which cells can be grown in a three-dimensional (3D) way. By studying these 3D tissues, such as mini organs (also called organoids) and organs-on-chips grown from human cells, the research directly translates to human health and saves animals from being experimented on. It is also often cheaper and faster.

Organoids

Lab-grown mini organs were first developed in 2013.⁴⁴ They are miniature versions of human organs and can be grown from many different organs. Healthy or tumour cells are taken via a small biopsy from a person's organ, bathed in a culture that stimulates them to grow over a few weeks or months and to organise themselves into mini versions of human organs.

For example, the process of growing a gut organoid has been described in three steps in the following way:

1. “Take a tissue sample. A very small biopsy is taken from the epithelium, the tissue lining the gut.
2. Incubate. The tissue is bathed in a mix of growth factors designed to let gut stem cells replicate.
3. Organoids, a millimeter or less in diameter, emerge in up to 3 weeks and can be frozen for later use”.⁴⁵

Organoids have many applications. They can be used for:

- **Regenerative medicine** – organoids grown from healthy tissue could be placed back into a patient to help repair damaged tissue.
- **Toxicity testing** – toxicologists can use organoids to test the effects of chemicals on the liver and other human organs.
- **Drug testing** – drugs can be tested on organoids to help predict their effects in patients.
- **Microbiome studies** – scientists can study how normal human intestinal bacteria interact with gut organoids.
- **Modelling infections** – organoids can be infected with viruses or bacteria to study how these affect cells.

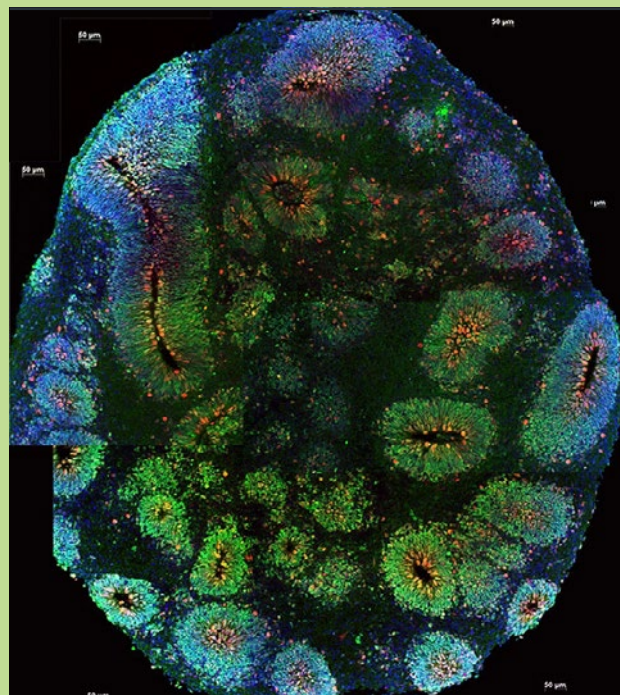
⁴⁴ Grens, 2018

⁴⁵ Sinha, 2017, p. 4

- **Personalised medicine** – organoids grown from individual patients can help predict their response to new or existing drugs.
- **Cancer studies** – scientists can study how cancer develops by introducing mutations in organoids grown from healthy tissues.⁴⁶

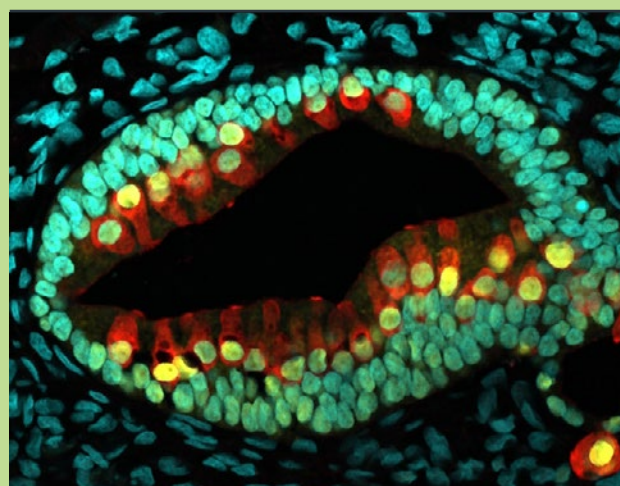
These mini organs are not capable of reproducing all biological responses like a real human organ, but they allow researchers to study a variety of physiological responses to specific manipulations and treatments. They allow for “surrogate” trials before conducting clinical trials, “providing a vital, physiologically relevant bridge between pre-clinical investigations and clinical outcomes and bringing the possibility of personalised medicine closer”.⁴⁷

Brain organoid



Source: National Institutes of Health, US⁴⁸

Inner ear organoid



Source: National Institutes of Health, US⁴⁹

⁴⁶ Sinha, 2017

⁴⁷ Archibald, Tsaioun, Kenna, & Pound, 2018, p. 2

⁴⁸ NIH Image Gallery, 2019

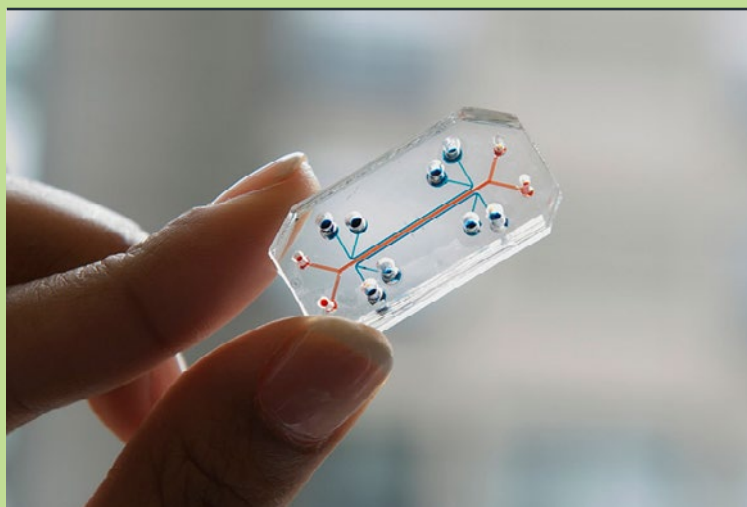
⁴⁹ NIH Image Gallery, 2018

Organs-on-chips

Mini organs can also be grown on microchips. Researchers have used microchip manufacturing methods to engineer microfluidic⁵⁰ culture devices that can mimic the structures and functions of living human organs.

These organs-on-chips are made of a clear and flexible polymer (molecules of a simple compound joined together) and contain hollow microfluidic channels lined with living human cells. Microfluidic channels contain tiny amounts of liquid ranging from submicron (smaller than one millionth of a metre) to a few millimetres. They are equipped with mechanical forces that can mimic the physical environment of organs, such as breathing motions (lung-on-a-chip) and peristalsis-like movements (intestine-on-a-chip). When nutrients, air, blood or drugs are added, the cells replicate some of the key functions of the organ. These organs-on-chips, in which cells can grow and fluids can flow, are usually the size of a computer memory stick.

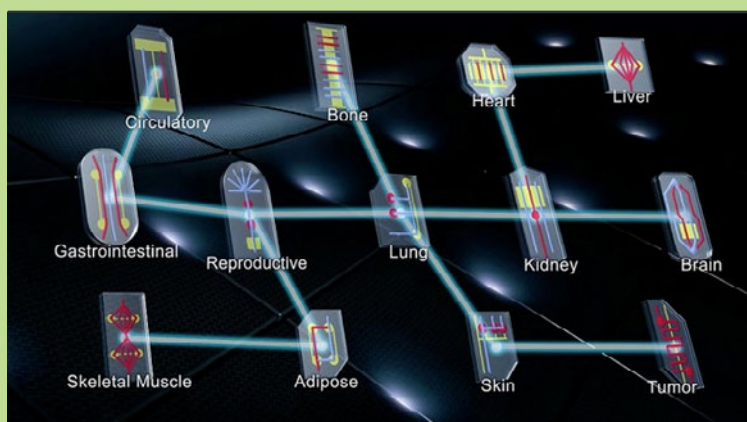
Lung-on-a-chip



This lung-on-a-chip serves as an accurate model of human lungs to test for drug safety and efficacy.

Source: *National Center for Advancing Translational Sciences, US*⁵¹

Human-body-on-a-chip



Multiple tissue chips can be connected in a system to simulate a human-body-on-a-chip.

Source: *National Center for Advancing Translational Sciences, US*⁵²

⁵⁰ At the micro scale, often flowing through channels.

⁵¹ National Center for Advancing Translational Sciences, no year-d

⁵² National Center for Advancing Translational Sciences, 2018d

The term organ-on-a chip was thought up by Donald Ingber, the Founding Director of the Wyss Institute for Biologically Inspired Engineering at Harvard University.⁵³ Together with his multidisciplinary team he developed in 2010 a lung-on-a-chip.⁵⁴ Professor Ingber is often credited with having developed the first organ-on-a-chip, but similar work had been undertaken some years earlier by a team from Seoul National University and Cornell University.⁵⁵

Organs-on-chips use different types of human cells: primary cells (cells directly taken from an organ or a tissue), immortalised cell lines (cells that have been modified by chemicals or a virus in order to survive and stay active indefinitely), or stem cells.⁵⁶

Organ-on-a-chip platforms that have already been developed include liver, skin, vascular structures (such as arteries, veins, capillaries), cardiac muscle, skeletal muscle, lung, bone and bone marrow, brain, eye, gut, spleen and kidney. There are also multi-organ-on-a-chip platforms that mimic the interplay between different organs.⁵⁷

A recent workshop attended by experts from industry, academia and the Medicines and Healthcare products Regulatory Agency (MHRA) held in Liverpool UK identified the advantages of organ-on-chip technologies:

“These dynamic and responsive biological test platforms have the potential to revolutionise drug target identification and validation studies without the need for animal models. This will improve compound efficacy, safety and targeted drug delivery.”⁵⁸

The experts also identified the technical, funding and regulatory challenges still to be overcome. Some of the technical challenges include physically-relevant cell interactions, scaling ratios between organs, and incorporation of immune or

endocrine systems. The capabilities and interactions in multiple organ-on-a-chip systems are only in the early stages of development. However, assuming future collaboration between organ-on-chip innovators, users, regulators and funders, the participants of this workshop anticipated real patient benefits by replacing poorly predictive animal models with these more physiologically relevant human-based models.

Organ-on-chip technology is still mostly used in-house by the companies and laboratories that developed it. However, researchers in academic institutes and biochemical, pharmaceutical, cosmetics and chemical companies have started to use the technology, and it can also be used by hospitals for personalised medicine. Researchers have also developed disease models, such as chips with tumour cells to study cancer.

“Chip-based in vitro organ models are ranked 6th among the top ten emerging technologies by the World Economic Forum in 2016, thus highlighting the potential of organ-on-a-chips to improve lives and transform the health care system. In other words, organ-on-a-chip technology is expected to speed up pharmaceutical drug development efforts, improve translation of basic research to clinically relevant patient scenarios and provide personalized intervention strategies.”⁵⁹

Organs-on-chips can replace many different species of animals currently used for research, such as mice, rats, rabbits, guinea pigs and non-human primates, which are all now used for drug testing and vaccine development. This technology has great potential for pharmaceutical research and personalised medicine. Apart from saving the lives of millions of animals, it is also cheaper and faster than animal experiments.

“Organs-on-chips are bio-engineered devices that mimic key aspects of the physiology and function of human organs, replicating some of the complexity of the human body environment on a microscopic scale. They are able to mimic blood, air and nutrient flow, as well as mechanical forces such as peristalsis and can be continuously monitored to obtain a profile over time. Organs-on-chips enable the study of basic biological processes, the modelling of diseases and investigation of the effects of drugs. They can potentially identify safety and efficacy issues earlier and more reliably in the drug development process, enabling the design and selection of drug candidates that are more likely to succeed in human clinical trials.”⁶⁰

Organ-on-chip technology is still quite new and there is little standardisation: “Each team is developing its own approach, with its own unique technology. The players are mainly start-up companies commercializing prototypes developed in the local universities.”⁶¹ It is a fast developing industry.



Organs-on-chips have great potential for personalised medicine, something not possible with animal testing.

⁵³ Zhang, et al., 2018

⁵⁴ Huh, et al., 2010

⁵⁵ T. H. Park & Shuler, 2003

⁵⁶ Ahadian, et al., 2018

⁵⁷ Ahadian, et al., 2018

⁵⁸ Haddrick, et al., 2018, p. 4

⁵⁹ Rothbauer, Rosser, Zirath, & Ertl, 2019, p. 84

⁶⁰ Archibald, et al., 2018, p. 2

⁶¹ Wilkinson, 2019, p. 648

Mini guts for personalised treatment of people with cystic fibrosis

Cystic fibrosis is a genetic condition that primarily affects the lungs and digestive system. There is currently no cure for the condition, which is caused by mutations in the CFTR gene that affect the function of the CFTR protein. There are drugs for the treatment of cystic fibrosis, but different drugs work for different mutations of the CFTR gene. Not all drugs work for all patients.

Researchers in the Netherlands have shown how these drugs could be tested for individual patients. They took rectal biopsies from patients, grew them into mini guts, and tested the available drugs for effectiveness. They showed that the drug responses observed in the mini guts could be used to predict which cystic fibrosis patient would respond to which drug. This test can help to quickly identify the best drug therapy even when patients carry very rare CFTR mutations.⁶²

Cystic fibrosis research has been carried out on different species of animals, for example, mice, pigs and ferrets.⁶³ But these “animal models” can’t predict which drug therapy will work for an individual patient with cystic fibrosis.

Blood-brain barrier on a chip

The blue dye shows where the brain cells would go, and the red dye shows the route for blood circulation.

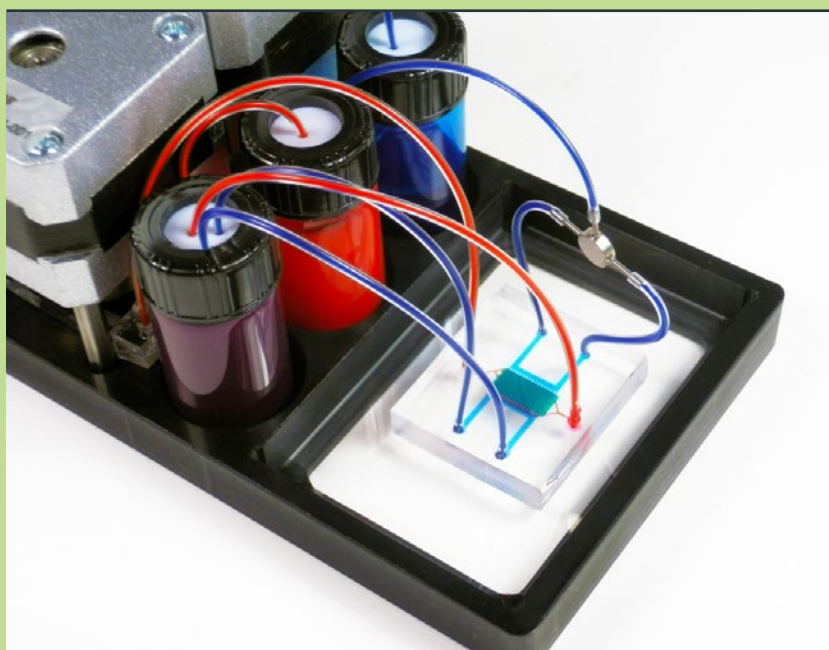
Source: National Center for Advancing Translational Sciences, US⁶⁶

Brain-on-a-chip on drugs

A brain-on-a-chip has been dosed with the street drug crystal meth (crystal methamphetamine, also called “ice”). Crystal meth is a stimulant that speeds up the messages moving between the brain and the rest of the body. Researchers from the US, Sweden and Israel connected three chips with different types of brain cells to model the blood-brain barrier and then added crystal meth to observe how the drug affects the brain. They were able to observe previously unknown interactions between blood vessels and neurons in the brain.

“The human brain, with its 100 billion neurons that control every thought, word, and action, is the most complex and delicate organ in the body. Because it needs extra protection from toxins and other harmful substances, the blood vessels that supply the brain with oxygen and nutrients are highly selective about which molecules can cross from the blood into the brain and vice versa. These blood vessels and their unique network of supporting pericyte and astrocyte cells comprise the blood-brain barrier (BBB). When the BBB is disrupted, as happens with exposure to drugs such as methamphetamine (‘meth’), the brain’s sensitive neurons become susceptible to harmful damage”.⁶⁴

Many different species have been used in brain research. While rats and mice are more likely to be used to study simple cognitive functions, non-human primates have been subjected to invasive brain research because scientists believe their brain processes are more similar to those of humans. With the development of brains-on-chips, many complex processes in the brain can now be studied without inflicting pain and suffering on animals. The new methods are also faster and cheaper.⁶⁵



⁶² Dekkers, et al., 2016

⁶³ Lavelle, White, Browne, McElvaney, & Reeves, 2016

⁶⁴ Brownell, 2018

⁶⁵ Maoz, et al., 2018

⁶⁶ National Center for Advancing Translational Sciences, no year-b

Placenta-on-a-chip

Scientists at a university in Vienna have created a placenta model on a microchip made up of two areas: one represents the mother, the other represents the foetus. A gelatin-based material was used to provide a structure to which human umbilical-vein endothelial cells⁶⁷ and cells from the placenta were added.

Many studies show that conditions of the mother, such as diabetes and high blood pressure, can have an effect on the unborn child. Compared with other research methods, this can now be studied in greater detail on the placenta-on-a-chip. The researchers who developed this chip intend to use it to study how nutrients such as glucose are transferred from the mother to the foetus, specifically in situations that involve a health risk for the foetus.⁶⁸

This placenta on a microchip models the human situation much better than animal experiments. It can also be used to study conditions in individual patients, something that is not possible with animal models.

Skin-on-a-chip

Microchips can test pharmaceuticals and cosmetics on human skin. A team in Singapore has developed a credit-card sized device that can overcome a limitation of traditional skin culture systems, which use cell cultures on a collagen matrix that easily shrinks. Instead, the team developed a new method that prevents skin contraction, using a fibrin⁶⁹-based matrix. Their microfluidic chip has several chambers. This allowed the researchers to grow skin in the device and conduct tests without having to transfer the skin.

The chip is made of thermoplastics⁷⁰ and can be mass produced. This skin-on-a-chip is suitable for high-throughput screening. The researchers wrote that it has “enormous potential to revolutionize many pharmaceutical, toxicological, and cosmetic applications, including safety and efficacy, which currently rely on animal testing”.⁷¹

Cosmetics have been tested on many species of animals, but perhaps best known is the Draize test. In 1944, John Henry Draize (1900-1992) and his colleagues at the US Food and Drug Administration (FDA) developed Draize rabbit irritation tests for identifying and evaluating toxic reactions when test materials are in contact with the skin, penis, and eyes. The tests were originally used for evaluating the safety of cosmetics and then extended to include insecticides, sunscreens and antiseptics.

Draize tests are painful and many animals are killed after the tests. These tests have been criticised because of large variations in test results and because of differences in the skin and eyes of humans and rabbits.⁷²



⁶⁷ Cells from the umbilical cord.

⁶⁸ Mandt, et al., 2018

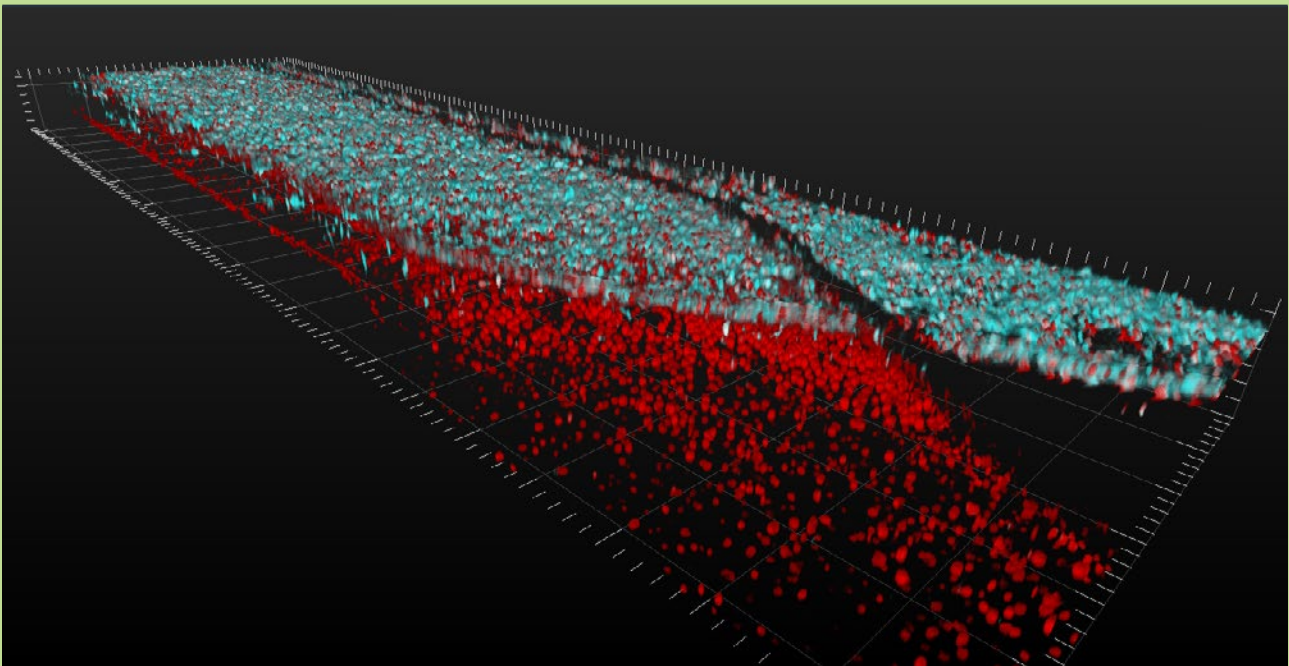
⁶⁹ Fibrin is a protein.

⁷⁰ Thermoplastics are types of plastic materials which become soft when they are heated and hard when they cool down.

⁷¹ Sriram, et al., 2018, p. 338

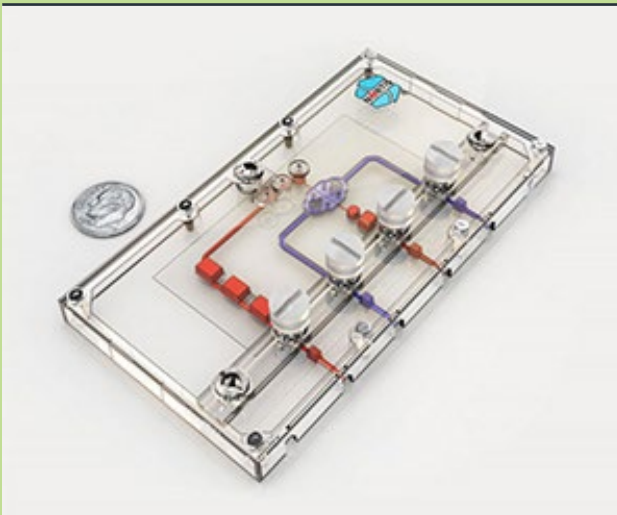
⁷² Lee, Hwang, & Lim, 2017

Spinal cord nerve and blood vessel cells on a tissue chip



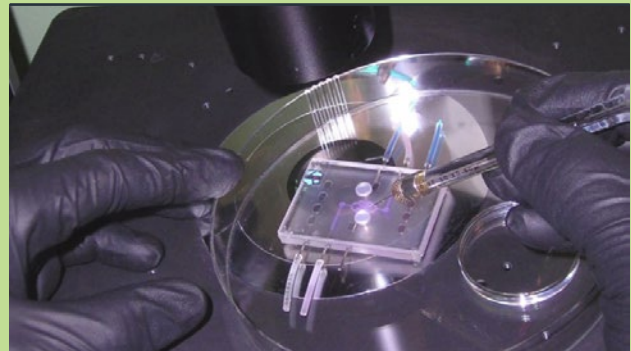
Source: National Center for Advancing Translational Sciences, US⁷³

Heart-on-a-chip (as compared to a dime)



Source: National Center for Advancing Translational Sciences, US⁷⁴

Kidney-on-a-chip



Source: National Center for Advancing Translational Sciences, US⁷⁵

⁷³ National Center for Advancing Translational Sciences, 2018f

⁷⁴ National Center for Advancing Translational Sciences, 2018c

⁷⁵ National Center for Advancing Translational Sciences, no year-c



Biobanking

To study human cells and tissues, researchers need a readily available supply of these human biological samples. They are stored in so-called biobanks, which are simply tissue banks. Biobanks use tissue left over from clinical procedures such as surgery and from dead bodies, or they collect tissue specifically for research. Biobanks also store organoids.

“Organoid biobanking is a promising and exciting new field with considerable potential for scientific research, precision medicine, and regenerative medicine”.⁷⁶

While biobanking has great potential, there are also obstacles:

“A number of key factors limit the wide adoption of non-animal human tissue models in cancer research, including deficiencies in the infrastructure and the technical tools required to collect, transport, store and maintain human tissue

for lab use. Another obstacle is the long-standing cultural reliance on animal models, which can make researchers resistant to change, often because of concerns about historical data compatibility and losing ground in a competitive environment while new approaches are embedded in lab practice”.⁷⁷

However, these obstacles can be overcome by improving infrastructure and collaboration. The collection of tissue also raises ethical issues, such as donor consent. Unless the tissue samples are used for personalised medicine, they are de-identified for biobanking. But complete de-identification may not always be possible and a de-identified sample can't be used to benefit the donor.

⁷⁶ Bredenoord, Clevers, & Knoblich, 2017

⁷⁷ Jackson & Thomas, 2017



'Omics' technologies

The term "omics technologies" refers to areas of study in biology whose names end in "omics", such as genomics. There are too many omics areas of study to list here. The most common omics include:

- **Genomics** – the field of science focusing on the structure, function, evolution, mapping, and editing of genomes.⁷⁸
- **Metabolomics** – the study of the set of metabolites⁷⁹ in an organism, cell, or tissue. It holds great promise for precision medicine. Small numbers of metabolites have been used for decades to diagnose metabolic diseases, such as the development of blood glucose test strips in the 1950s to test for diabetes. With metabolomics, much larger numbers of metabolites than are presently covered in standard clinical laboratory techniques can be examined.⁸⁰ This allows for personalised diagnosis and treatment of individual patients.
- **Proteomics** – the large-scale study of proteins, particularly their structures and functions.
- **Transcriptomics** – the study of messenger RNA (ribonucleic acid) molecules produced in an individual or population of a particular cell type.⁸¹

Recently, a number of beliefs about our understanding of human biology have been called into question following studies using omics approaches. Such beliefs include the idea that the genome is static throughout an organism's lifetime, that it is identical in all cell types, and that all of the necessary information for cellular function is contained within the gene sequence.⁸²

"Traditional toxicology evaluates end points such as death, disease or observable changes in the organism or cells of the organism, while 'omics' measurements are made across multiple levels of biological organisation and provide information that may be used to understand cellular processes as an integrated system rather than as a collection of disparate measurements".⁸³

Using several of these omics approaches together can help scientists learn about toxicity pathways in human cells and study the underlying cause of disease.

"The growing use of 'omics' technologies (e.g. transcriptomics, proteomics and metabonomics) in combination with in vitro test systems allows a comprehensive analysis of the impact of a chemical at the molecular level and can indicate potential toxicity pathways that may lead to adverse health effects".⁸⁴

Metabolomics approach to identify gum disease in its early stages

Periodontitis is an advanced gum disease that involves inflammation of the gums and the supporting structure of the teeth. If untreated, it leads to the loss of teeth. The condition is very common and often diagnosed late when substantial damage has already occurred. It is preventable when diagnosed early.

A group of researchers in France tested saliva from people with and without periodontitis, using nuclear magnetic resonance (NMR) spectroscopy together with multivariate statistical analysis to identify the metabolic signature of active periodontitis.⁸⁵ They looked for a range of metabolites and found that a combination of lactate, GABA, and butyrate predicted the presence of periodontitis. The study showed that this simple and non-invasive method could be used for early diagnosis and follow-up of periodontitis.

Rats, mice, hamsters, rabbits, ferrets, sheep, pigs, cats, dogs and non-human primates have been used for modelling human periodontal diseases and treatments. Of these animal species, non-human primates and dogs are considered to be closest to humans in their anatomy and the way in which dental disease develops.⁸⁶ This new method developed by the researchers in France is more accurate than animal models.

⁷⁸ A genome is the genetic material of an organism.

⁷⁹ Metabolites are small molecules necessary for metabolism.

⁸⁰ Clish, 2015

⁸¹ Our genetic material is encoded in DNA. RNA is similar to DNA, but has another function: it communicates genetic information to the rest of the cell.

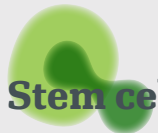
⁸² McBride, 2017

⁸³ McBride, 2017, pp. 69-70

⁸⁴ European Commission, 2018b

⁸⁵ Rzeznik, et al., 2017

⁸⁶ Struillou, Boutigny, Soueidan, & Layrolle, 2010



Stem cell technologies

When cells are used to test drugs or other substances, it is important that they are reliable representatives of cells or cellular systems in the human body. Many traditional in-vitro methods that have been used for decades, such as growing cells in a two-dimensional way on a Petri dish, do not meet these criteria as they are using immortalised cell lines or isolated primary cells.

Immortalised cells⁸⁷ can be grown indefinitely and they are easily cloned and cost effective, but they are genetically altered and may react in different ways to cells that have not been altered. Isolated primary cells are cells from human or animal tissue. They are of varying quality and may not react in a consistent fashion.⁸⁸ Stem cells are a promising alternative source of human cells for toxicity testing, studying and treating disease.

Stem Cells Australia, an initiative that links Australia's leading experts in bioengineering, nanotechnology, stem cell biology, advanced molecular analysis and clinical research, explained these technologies in the following way:

“What type of stem cells are there?”

Stem cells can be divided into two broad groups: tissue specific stem cells (also known as adult stem cells) and pluripotent stem cells (including embryonic stem cells and iPS cells). Tissue specific stem cells are derived from, or resident in, adult tissues, and usually only give rise to the cells of that tissue, thus they are considered

multipotent. Embryonic stem cells, derived from a small group of cells in the early embryo (5-7 days), and iPS cells are undifferentiated and are considered pluripotent as they can become every type of cell in the body.

What are induced pluripotent stem cells (iPS)?

Recently, scientists discovered that a mature fully specialised cell, for example a human skin cell, in the right conditions could be induced to mimic the characteristics of an embryonic stem cell. These are known as induced pluripotent stem cells (iPS cells).⁸⁹

Induced pluripotent stem cells were first developed in 2006 through genetic reprogramming of adult cells.⁹⁰ The Nobel Prize in Medicine in 2012 was awarded for the discoveries that cells can be reprogrammed to become pluripotent stem cells, and that cells from individual patients could be harvested and reprogrammed to become any tissue type found in the human body.⁹¹

The use of stem cells has great potential for studying and treating diseases.⁹² In theory, there is no limit for the type of diseases that could be treated with stem cell methods. However, new methods have to be tested first to ensure they are safe and effective. Some stem cell therapies are already being used in cancer treatments and bone marrow transplantation. Human stem cells are also now being used to test drugs.

Parkinson's disease identified as a possible autoimmune disease

A group of researchers at a German university has gained new insights into the development of Parkinson's disease by using a stem cell approach. The researchers had observed an unusually high number of T cells⁹³ in the midbrain and the blood of Parkinson's patients. These T cells – a type of T cell similar to those found in people with autoimmune diseases – attack and kill nerve cells that produce dopamine in the midbrain.

With this observation in mind, the researchers took small skin samples from healthy people and people with the disease, and differentiated these cells into stem cells that function like midbrain nerve cells. These were then brought into contact with fresh T cells from the people who had donated the skin samples. The researchers found that the stem cells from people with Parkinson's disease killed a large number of their nerve cells, but healthy people's cells did not react in this way.⁹⁴

⁸⁷ cells that continue to divide indefinitely

⁸⁸ McBride, 2017

⁸⁹ Stem Cells Australia, 2018

⁹⁰ Brevini, et al., 2016

⁹¹ Rothbauer, et al., 2019

⁹² Triunfol, et al., 2018

⁹³ T cells are a type of white blood cells.

⁹⁴ Friedrich-Alexander Universitaet Erlangen-Nuernberg, 2018; Sommer, et al., 2018

Human stem cells could potentially be used as a renewable source of replacement cells and tissues to treat diseases such as macular degeneration, spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis and rheumatoid arthritis.⁹⁵ Stem cell banks are being established worldwide and the global market for human-induced pluripotent stem cell technology looks promising.⁹⁶

The use of embryonic stem cells has raised questions about the ethics of their use, as each embryonic stem cell line has been grown from a human embryo created through in-vitro fertilisation (IVF) or through cloning technologies.

EBiSC

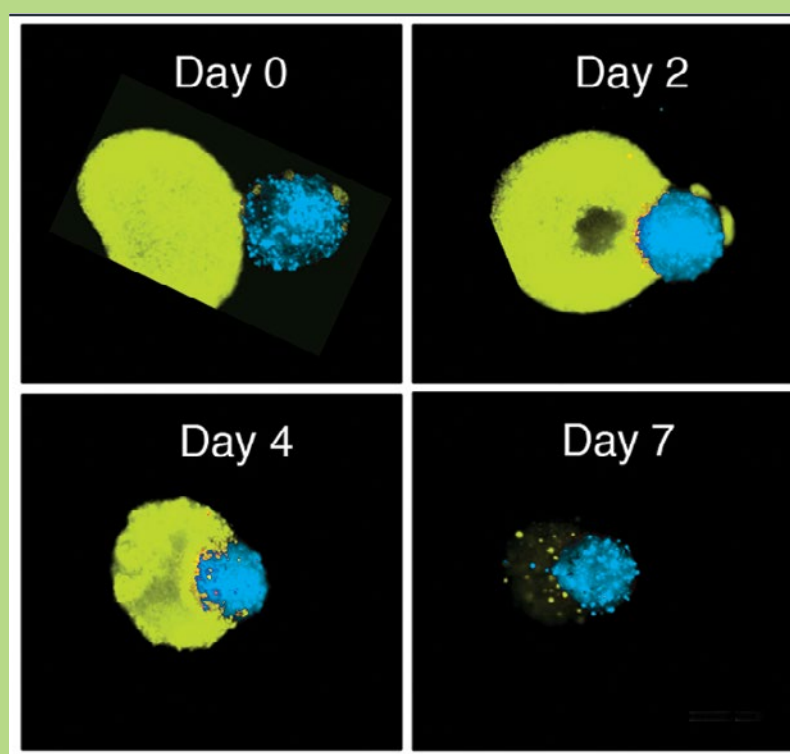
In Europe, a large public-private partnership project has set up biobanks for induced pluripotent stem (iPS) cells, the European Bank for induced pluripotent Stem Cells (EBiSC).⁹⁷ EBiSC anticipates that its capacity will be 10,000 cell lines. At the time of writing, EBiSC's online catalogue included more than 800 different cell lines and an additional 240 were in the process of being tested for quality.

The use of human stem cells can replace many experiments on animals, in particular “humanised mouse models”, that is mice that have been engrafted with human cells and tissues to give them human diseases. Although scientists have been able to cure cancer in mice for decades⁹⁸, these animals are still being used to study human cancers.

Neural stem cells in 3D culture models

Scientists at the University of North Carolina at Chapel Hill (UNC) determined the anti-tumour effects of neural stem cells⁹⁹ in 3-D culture models. Stem cell aggregates, or spheroids (blue in the images below), were placed next to brain cancer cell spheroids. Fluorescent images captured over seven days showed that the stem cell therapy decreased the volume of cancer spheroids. The researchers are working to advance the therapy towards first-in-human clinical trials.

Neural stem cells in 3D culture models



Source: National Center for Advancing Translational Sciences, US¹⁰⁰

⁹⁵ National Institutes of Health, 2016

⁹⁶ Archibald, et al., 2018

⁹⁷ European Bank for induced pluripotent Stem Cells, 2018

⁹⁸ Cimon, Getlin, & Maugh, 1998

⁹⁹ Neural stem cells are cells that generate the neurons and glia of the nervous system during embryonic development.

¹⁰⁰ National Center for Advancing Translational Sciences, 2018e

Glomerulus chip with stem cells to study kidney disease

Researchers from the Wyss Institute for Biologically Inspired Engineering at Harvard University have developed a protocol to grow kidney cells from induced pluripotent stem cells (iPS cells) within a microfluidic organ-on-a-chip to build a human kidney glomerulus chip that mimics the structure and function of the kidney glomerular capillary wall. The glomerulus chip can be used to study human kidney development, function and disease. It has the potential to be used in regenerative medicine as a cell therapy for people with kidney diseases.¹⁰¹

Using induced pluripotent stem cells to understand the development of cancer in children

Reprogramming of human cells that can be easily obtained from biopsies or blood samples to induced pluripotent stem (iPS) cells can help as a prognostic tool and to discover familial cancers early. Patient-derived iPS cells from a tumour can also help develop specific drugs for that individual child.¹⁰²

“The discovery of iPS cells opens up a wide spectrum of possible future applications including development of new treatments in regenerative medicine, generation of better and more accurate disease models, and improving drug discovery. Where other tools fail, using patient-specific iPS cells for modeling diseases have an incredible potential to improve our understanding of basic mechanisms operating during healthy and diseased human development and differentiation”.¹⁰³

Human pluripotent stem cells for modelling liver development and disease

Pluripotent stem cells can help scientists understand liver disease much better than traditional methods, such as studies using mice. Mice are different from humans in diet, genetics, gene expression and physiology. Mouse studies require time and are unsuitable for high-throughput methods. In contrast, iPS cells can be expanded indefinitely and differentiated into liver-like cells that have many functional characteristics of human livers. Mutations can be introduced through genome engineering to help researchers study liver development or to model rare liver diseases. Studying liver disease, such as hepatitis A and C, and disease development using iPS cells can be undertaken with semi-automated, high-throughput methods that make it possible to screen a wide range of pathways and functions simultaneously.¹⁰⁴

¹⁰¹ Musah, Dimitrakakis, Camacho, Church, & Ingber, 2018

¹⁰² Navarro, Susanto, Falk, & Wilhelm, 2018

¹⁰³ Navarro, et al., 2018, pp. 7-8

¹⁰⁴ Heslop & Duncan, 2018

3D and 4D bioprinting

Bioprinting, or tissue printing, is one of the technologies of tissue engineering and regenerative medicine (TERM), which involves researchers from different disciplines, such as medicine, engineering, biology and chemistry. In recent years, they have achieved much progress in TERM.¹⁰⁵

Three-dimensional (3D) printing was first conceived in 1986 and has since influenced fields such as engineering, manufacturing and medicine.¹⁰⁶ While there are different technologies of 3D printing, it basically involves processes where materials or liquids are joined, layer by layer, under computer control, to create three-dimensional objects.

The terms 3D printing and 3D bioprinting have different meanings. Both processes build a 3D object

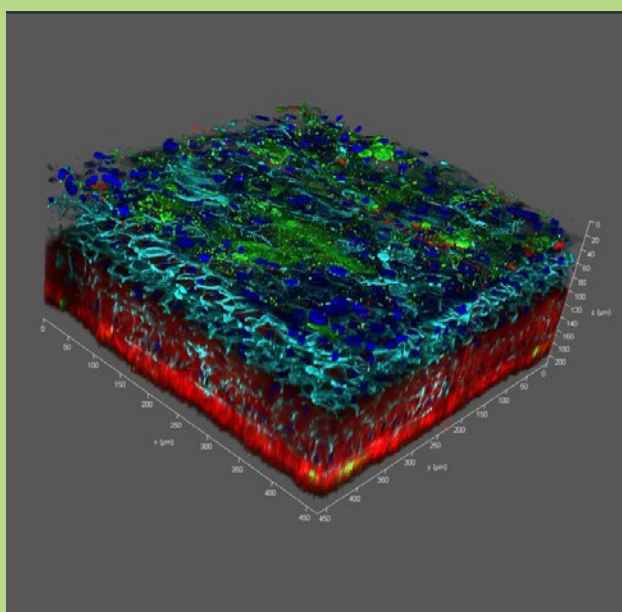
layer by layer from a 3D model but “3D bioprinting involves the use of cell-laden bioinks and other biologics to construct a living tissue while 3D printing technologies do not use cells or biologics”.¹⁰⁷

3D printing technologies were originally designed for non-biological applications, such as metals, ceramics and thermoplastic polymers. The process used organic solvents, high temperatures or materials that are not compatible with living cells and biological materials. A challenge for TERM scientists then, is to keep the cells alive during the printing process. For 3D bioprinting, the researchers had to find materials and printing processes that are compatible with living cells and tissues.¹⁰⁸ They also had to find suitable materials to build scaffolds that contain

and shape the biomaterials in a desired form. The scaffold materials can be natural or synthetic.

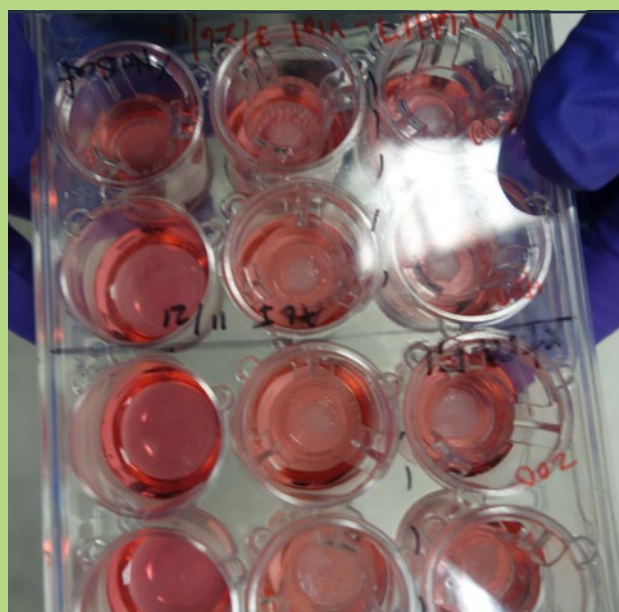
“Bioprinting is no longer confined to a process for combining one cell type with one material; the emphasis today is to use a variety of material types to create bespoke scaffolds onto which chemical cues can be tethered and multiple cell types can be printed with precision”.¹⁰⁹

3D image of bioprinted skin tissue



Source: National Center for Advancing Translational Sciences, US¹¹⁰

3D printed eye tissue



Source: National Center for Advancing Translational Sciences, US¹¹¹

¹⁰⁵ K. M. Park, Shin, Kim, & Shin, 2018

¹⁰⁶ Bishop, et al., 2017

¹⁰⁷ Vijayavenkataraman, Yan, Lu, Wang, & Fuh, 2018, p. 2

¹⁰⁸ Murphy & Atala, 2014

¹⁰⁹ Mehrban, Teoh, & Birchall, 2016, p. 13

¹¹⁰ National Center for Advancing Translational Sciences, 2018a

¹¹¹ National Center for Advancing Translational Sciences, no year-a

Researchers have developed many different approaches to 3D bioprinting, including:

- **Laser-based bioprinting** – laser energy is used to pattern bioinks laden with cells.
- **Droplet-based bioprinting** – cell-laden bioinks are ejected out of the nozzle in the form of droplets.
- **Extrusion-based** – bioink is pushed out of the nozzle using pneumatic pressure or mechanical force by means of a piston or screw. This is the most widely used type of bioprinting. Extrusion-based bioprinting has been used to bioprint cells, tissues and organ-on-a-chip devices for tissue engineering, cancer research, drug testing and transplantation.
- **Stereolithography bioprinting** – where a “layer of photopolymer resin is cured (or polymerized) by light (usually UV) irradiation, the light

movement controlled by a computer code/images/CAD files, forming a 3D structure as the build stage is translated vertically building the object layer by layer.”¹¹²

“In laser-based writing, which consists of a laser beam, a substrate, and a focusing system, cells are confined in a laser beam and deposited in a steady stream on nonabsorbing surfaces, including biological gels. As such, cells can be printed continuously and accurately without causing significant cell death. Laser-based writing can pattern cells with high resolution up to the micrometer scale and is advantageous over other bioprinting techniques as it can be used with many materials and does not directly bring the live cells into contact with the substrate, improving cell survival.

This technique has been used to produce vascular networks with micrometer precision on biological gels in vitro”.¹¹³

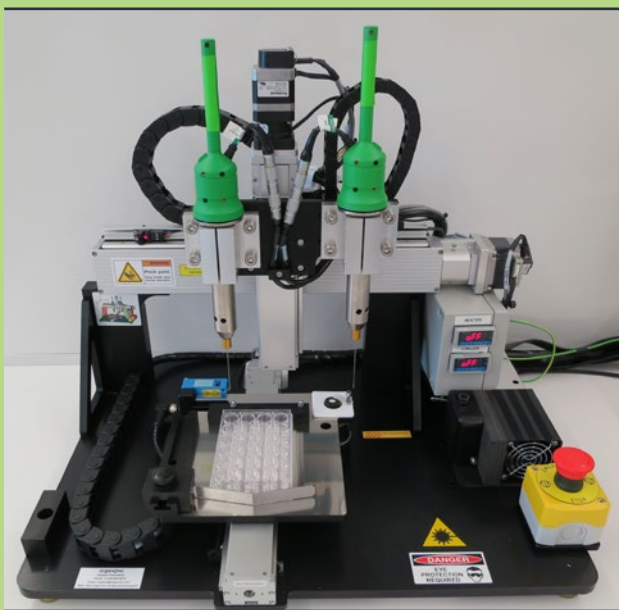
A disadvantage of laser-based bioprinting is its slow printing speed which makes this method unsuitable for printing of large tissues or organs.

“In inkjet printing, which consists of a reservoir tank, an orifice, and a print head, a pressure is created in the tank, which pushes the ink in the orifice and out to the printer head. As a result, cell droplets are deposited on a surface, which provides the advantage of limiting contact of cells and materials on a surface. Other advantages of inkjet printing include controllable resolution, high printing speed, and relatively low material costs”.¹¹⁴

Inkjet printing is the most popular printing method. It is fast, cheap and readily available. The disadvantages include print-head clogging, mechanical stress and unreliability in bioink dispensing.¹¹⁵

“Microextrusion printers use pneumatic or mechanical (piston or screw) dispensing systems to extrude continuous beads of material and/or cells”.¹¹⁶ This method has already been used to produce, for example, aortic valves¹¹⁷ and an ovarian cancer disease model.¹¹⁸ But it has disadvantages, too. They include limited spatial resolution and, as high pressures are used, part of the living material may not survive the printing process.¹¹⁹

Organovo bioprinter



Source: National Center for Advancing Translational Sciences, US¹²⁰

¹¹² Vijayavenkataraman, et al., 2018, p. 11

¹¹³ Ahadian, et al., 2018, p. 19

¹¹⁴ Ahadian, et al., 2018, p. 20

¹¹⁵ Mehrban, et al., 2016

¹¹⁶ Murphy & Atala, 2014, p. 775

¹¹⁷ Duan, Hockaday, Kang, & Butcher, 2013

¹¹⁸ Xu, et al., 2011

¹¹⁹ Mehrban, et al., 2016

¹²⁰ National Center for Advancing Translational Sciences, no year-e

A research group from Singapore described the typical steps involved in bioprinting in the following way:

“A typical bioprinting process consists of three major steps namely pre-processing, processing and post-processing. Preprocessing involves imaging of the tissue or organ using computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound imaging techniques and reconstruction of 3D models from the imaging. The generated 3D models are then converted into STL file format, which is a commonly accepted file format by most of the commercially available bioprinters. The processing step starts with harvesting primary cells from patients, culturing and expanding it ex vivo for the bioprinting process. Though cancer cell lines and other non-human cells are being used, the ideal condition for fabricating transplantable living tissues would be to use the patient’s own cells. Suitable bioinks with properties mimicking the intended tissue to be printed are selected and the cells are suspended in these bioinks. The cell-laden bioinks are then fabricated into required 3D living tissue/organ according to the 3D model using a bioprinter. Post-processing involves maintaining the bioprinted tissue/organ in a bioprinter for tissue maturation before being transplanted into patients or used as in vitro models for disease modelling, or drug testing”.¹²¹

Different cell types can be used for bioprinting. Stem cells are particularly appealing “as they are pluripotent and able to differentiate into other cell types upon exposure to the correct physical and chemical guidance cues. Within the human body, there are a number of viable sources of stem cells, such as the bone marrow, periosteum and adipose tissue”.¹²²

Bioprinting has many applications in biomedical research and the health-care sector, such as disease modelling, drug discovery and testing, high-throughput screening and regenerative medicine. Bioprinting has already contributed to much progress in skin and cartilage repair.¹²³

“Advanced biofabrication techniques, such as 3D printing and 3D microfabrication can also be applied for the creation of tissue models, and organoid technologies can explore stem cells for the formation of organ- like structures to accurately and simultaneously capture multiple aspects of human physiology”.¹²⁴

“Bioprinting has a great potential to solve this ever-increasing organ shortage crisis. Though bioprinting of fully functional organs has a long way to go, considerable progress has been made to realize the greater goal of organ printing. Bioprinted tissues could be used as in vitro testing beds in place of animal testing. Given the ethical concerns surrounding animal testing and the high cost involved, bioprinting is a viable alternate. In pharmaceutical research, bioprinting could be used as in vitro models for testing of drug efficacy, toxicity, chemotherapy or chemo-resistance to reduce the high cost and shorten the time of drug discovery”.¹²⁵

Current 3D bioprinting technologies have several limitations. Some techniques don’t allow for the mixing of different types of cells or make such mixing very difficult, for instance, high temperatures or voltages may compromise the living cells or tissues. “Thus, there is always a trade between resolution, compatibility with cell deposition, cell viability as well as mechanical stability and no one of the existing 3D printing/ bioprinting methods is able to provide all advantages”.¹²⁶

4D printing, which is a more recent development, can overcome some of the challenges associated with 3D printing. 4D bioprinting has been described as 3D bioprinting with the added dimension of time¹²⁷, or shape transformation over time.¹²⁸

The new printing technique has been defined as 3D printing of cell-laden materials in which the printed structures would be able to respond to external stimulus or internal cell forces.¹²⁹ Simply, 4D bioprinted materials are capable of changing their shape over time. While 4D printing is still in its infancy, it has great potential for tissue engineering in many areas. The current demand for donor organs to replace damaged or lost organs is higher than the availability of donor organs, for instance, and 4D printing could one day provide individualised organs or part-organs to fulfil that demand. Current attempts to genetically engineer animals such as pigs to become organ donors for humans will then be redundant.

“The significantly enhanced usability and functionality of the bioprinted objects due to their capability to transform with time will likely find widespread applications in areas including but not limited to tissue engineering, regenerative medicine, bioelectronics, robotics, actuators, and even medical devices”.¹³⁰

¹²¹ Vijayavenkataraman, et al., 2018, p. 3

¹²² Mehrban, et al., 2016, p. 12

¹²³ Mehrban, et al., 2016

¹²⁴ Zhang, et al., 2018, p. 258

¹²⁵ Vijayavenkataraman, et al., 2018, p. 2

¹²⁶ Ionov, 2018, p. 1

¹²⁷ Ashammakhi, et al., 2018

¹²⁸ Li, et al., 2017

¹²⁹ Ashammakhi, et al., 2018

¹³⁰ Li, et al., 2017, pp. 4-5

A 3D bioprinted vascularised liver tissue model for drug testing

An international collaboration of researchers has developed a 3D bioprinted a vascularised liver tissue model that allows them to mimic drug diffusion and accurately test whether a drug is toxic to humans. A vascularised tissue is a tissue with vessels, especially blood vessels. Drug diffusion refers to the movement of a substance or drug from an area of high concentration to an area of lower concentration. Diffusion is an important process in living organisms. It occurs in liquids and gases, and allows substances to move in and out of cells. The researchers noted that this model could be expanded and used to model other organs for drug testing, such as the heart, kidney and bone.¹³¹

Bioprinting can and has already been used in combination with organs-on-chips. Although it is difficult to recreate complex bodily functions, the models that are created with organ-on-chip and bioprinting technologies allow for drug testing and the study of physiological functions in-vitro. The combination of both technologies can build tissues faster than organ-on-chip technology alone. It creates high throughput in the production of organs-on-a-chip, and it helps researchers to develop new types of organ-on-a-chip.¹³²



Robotic testing

Researchers, particularly those in the pharmaceutical industry, have developed automated methods to test biological activities of thousands of chemicals that used to be tested in animals. This is called high-throughput or robotic testing.

“The last two decades have seen innovations in technology that have helped to evolve automated, microprocessor controlled robotic processes called High Throughput Screening (HTS). This qualitative leap in drug discovery paradigm has been achieved via a synergy of chemistry, biology, engineering and informatics”.¹³³

In the US, high-throughput testing is being supported by a government initiative called Toxicology in the 21st Century (Tox21). It is a collaboration of several government agencies that aims “to develop better toxicity assessment methods to quickly and efficiently test whether certain chemical compounds have the potential to disrupt processes in the human body that may lead to negative health effects”.¹³⁴

“In the United States, Tox21 is a multi-agency collaboration to research, develop, validate, and translate chemical testing methods in order to characterize chemical toxicity pathways in [sic] interest of protecting public health. This initiative became official through the establishment of a Memorandum of Understanding between four major agencies: (1) the US Environmental Protection Agency (EPA)/Office of Research and Development, (2) the US National Institutes of Health (NIH), National Institute of Environmental Health Sciences/National Toxicology Program, (3) the NIH/National Human Genome Research Institute/Chemical Genomics Center, and (4) the US Food and Drug Administration (FDA). A central component of this initiative is to employ high throughput screening assays and genome analytical methods to identify mechanisms of chemical induced biological activity, prioritize over 10 000 chemicals for more extensive toxicological evaluation, and develop computational predictive models of in vivo biological response. ... The major objective of Tox21 is to deliver biological activity profiles that are predictive of in vivo toxicities for the thousands of substances that are being studied over the 5-year collaboration”.¹³⁵

¹³¹ Massa, et al., 2017

¹³² J. Y. Park, Jang, & Kang, 2018

¹³³ Ranganatha & J, 2012, p. 30

¹³⁴ United States Environmental Protection Agency, 2018b

¹³⁵ Valerio, 2014, pp. 1026-1027

The US Environmental Protection Agency (EPA) contributes to Tox21 through its computational toxicology research (CompTox), which can evaluate chemicals for potential risks quickly and at a small cost. These high-throughput screening assays can outperform the predictive ability of models built with animal toxicity data.¹³⁶ A key part of EPA's CompTox is its Toxicity Forecaster (ToxCast). Data generated by ToxCast are publicly available online. They complement the European Chemicals Agency (ECHA) database and contain "results from several thousands of in vitro tested chemicals, measuring hundreds of endpoints each".¹³⁷ ToxCast is a useful resource to inform read-across.^{138 139}

"Tox21 has established a library of ~10,000 chemicals for the production phase of the program, including the NCATS Pharmaceutical Collection (NPC), which contains drugs used in the clinic. This library has been screened against 47 cell-based assays in a quantitative high-throughput screening (qHTS) format generating nearly 70 million data points to date".¹⁴⁰

The Deepwater Horizon oil spill in the Gulf of Mexico and ToxCast

The 2010 BHP oil disaster in the Gulf of Mexico was the largest marine oil spill in the history of the petroleum industry. To break up the oil slick, more than 1.5 million gallons of the oil spill dispersant Corexit 9500 was used. Very little was known about the effects of the dispersant chemicals on acute or long-term toxicity in humans or marine animals. When the US EPA's Office of Research and Development was asked to evaluate the potential toxicity of oil spill dispersants, the researchers needed a fast testing method. They decided to use high-throughput in-vitro tools that are part of the EPA's ToxCast program.¹⁴¹ No animals were used in these tests. Indeed, animal tests could not have provided such quick and low-cost results.

"... we were able to detect specific bioactivities in complex chemical mixtures for time-sensitive environmental issues and using high throughput screening assays. This is exciting given that one of the challenges of real world chemical toxicity testing is the fact that humans and other organisms are often exposed to complex mixtures, rather than the pure single compounds that are the subject of typical toxicity testing. The in vitro tests used in this study rapidly profiled the complex dispersant formulations without the use of animals, and screened for potential endocrine activity, other endpoints and cytotoxicity. In different circumstances, a similar rapid screening effort could be used to make time-sensitive decisions based on potential hazard and risk".¹⁴²

Testing of nanomaterials is an area that would greatly benefit from high-throughput testing. Manufactured nanomaterials (materials with at least one dimension <100 nm¹⁴³) and nanoparticles (all three dimensions <100 nm) are used in many consumer products, but their effects on human health are not well known. While some high-throughput testing methods of nanomaterials are in development, they have not yet been validated.¹⁴⁴

High-throughput testing is also used with organs-on-chips. This technology is at present still too expensive for widespread use but work is underway to make its pricing more competitive so that it can replace conventional in-vitro and in-vivo models.¹⁴⁵

¹³⁶ Archibald, et al., 2018

¹³⁷ Maertens, Hubesch, & Rovida, 2016

¹³⁸ Chesnut, et al., 2018

¹³⁹ See section on in-silico methods for detail on read-across.

¹⁴⁰ R. Huang, et al., 2018, p. 2

¹⁴¹ Judson, et al., 2010

¹⁴² Judson, et al., 2010, p. 5984

¹⁴³ One nanometre (nm) is equal to one billionth of a meter.

¹⁴⁴ Collins, et al., 2017

¹⁴⁵ Probst, Schneider, & Loskill, 2018; van de Burgwal, van Dorst, Viëtor, Luttge, & Claassen, 2018

In-silico methods

Progress in information technology has enabled the development of computer-based (in-silico¹⁴⁶) methods for biomedical research and the testing of chemical and biological substances. To date, most progress with in-silico methods has been made in the area of toxicology (the study of the adverse effects of chemical substances on living organisms). Many computational methods have been developed to predict toxicity, and can thus play a role in replacing and/or reducing animal testing. The main prediction methods are described in this section. Computer modelling of health and disease is another area where in-silico methods can replace the use of animals.

“Chemical products are used in making 95% of all goods”.¹⁴⁷ Chemical and biological substances, such as newly developed drugs, need to be tested to determine whether they are harmful to humans, animals or plants. But there is limited information about most substances because testing is very expensive. The US Toxic Substance Control Act inventory includes 83,000 chemicals, but has data for only 3% of these.¹⁴⁸ Safety testing reportedly costs US\$10 – 20 million per product and takes several years. It is also difficult to obtain toxicological data for the 1,000 new chemicals created every year.¹⁴⁹



Computer-based testing and research methods are booming in the toxicology sciences.

The development of new drugs and pesticides is lengthy and extremely expensive, as the following statements show:

“Therapeutic development is a costly, complex and time-consuming process. The average length of time from target discovery to approval of a new drug is about 14 years. The failure rate during this process exceeds 95 percent, and the cost per successful drug can be \$1 billion or more”.¹⁵⁰

“The probable range confronting developers of new pesticide chemicals appears to be \$750,000 to \$3.25 million – but the trend is constantly upward. On average it costs €2.2 billion and takes 10 years for a new active substance to be brought to market according to the European Trade Association”.¹⁵¹

It is opportune, then, that computers can help researchers in the safety sciences by performing large amounts of complex calculations at great speed to reveal patterns, trends and associations. In silico methods have many applications, as a scientist from the US Food and Drug Administration observed:

“In silico methods for toxicology apply modern computing applications and informatic technologies as scientific tools to advance and gain efficiency to improving our understanding of toxicity potential, pathways, hazards, and risks of chemical and biological substances. Multiple computer technologies and methodologies serve to store, interface, process, or transmit information which enable scientific advancements in the toxicological sciences through implementation of predictive models, databases, detection systems, and simulations processes”.¹⁵²

The in-silico prediction methods for the evaluation of toxicity outlined below cover the most common methods and models but they do not represent a comprehensive list. Several organisations and companies have produced software packages for predicting toxicity or physicochemical properties of chemicals. In general, they contain one or more predictive models. Rapid progress is being made in this area and software, models and datasets are being constantly updated.¹⁵³

¹⁴⁶ In silico – performed on a computer or via computer simulation.

¹⁴⁷ Meigs, et al., 2018, p. 286

¹⁴⁸ Maertens & Hartung, 2018

¹⁴⁹ Chesnut, et al., 2018

¹⁵⁰ National Center for Advancing Translational Sciences, 2018b

¹⁵¹ Meigs, et al., 2018, p. 289

¹⁵² Valerio, 2014, p. 1026

¹⁵³ Myatt, et al., 2018

The *silico* methods for predicting and evaluating the toxicity of chemical and biological substances described in this section include:

- Structure-activity relationship (SAR) and quantitative structure-activity relationship (QSAR) modelling
- Read-across
- Physiologically-based pharmacokinetic (PBPK) models
- Expert systems

This is followed by a selection of tools:

- Read-across structure activity relationships (RASAR) modelling
- REACHacross
- Toxtree
- Toxmatch

There are also some approaches that are not considered a method or a tool:

- Adverse Outcome Pathways (AOPs) - a conceptual approach to representing knowledge about toxicity mechanisms
- Integrated Approaches to Testing and Assessment (IATA)

Lastly, this section provides an overview of computer modelling for the study of human organs and the body, and how this can be used in clinical settings¹⁵⁴. The use of computers for virtual reality and surgical simulation, mainly for purposes of education and training, will be covered in the section on simulators.

Computer-based approaches for the study of Parkinson's disease

Parkinson's disease (PD) is an age-related neurodegenerative disease whose cause or causes are largely unknown and for which a cure has not yet been found. During the 1950s, it was discovered that the psychiatric drugs reserpine and haloperidol induce Parkinsonian-like symptoms in humans. That discovery led to the drugs becoming some of the first used to develop animal models. That is, these drugs were given to animals to simulate Parkinson's disease. Decades and many animal models later, no animal model can fully explain the disease in humans:

“More than 60 years have been spent attempting to produce PD-like symptoms in various animal species as a model to study disease pathogenesis and treatment. In addition to rodents and nonhuman primates, *Caenorhabditis elegans* (roundworms), *Drosophila melanogaster* (fruit flies), fish (zebrafish and goldfish), and anurans (frogs and toads) have been used. However, each animal model generally demonstrates only specific subsets of PD characteristics, and no single model recapitulates all the known pathological processes associated with PD in humans”.¹⁵⁵

New approaches are needed that focus on humans, not on animals. These include *in-vitro* and *in-silico* approaches. *In-vitro* approaches consist of, for example, methods that use stem cells. *In-silico* approaches include computer simulations, mathematical algorithms and machine learning to predict interactions between molecules and pathways within biological systems.¹⁵⁶

Much information about Parkinson's disease exists already, for example in the Organization for Economic Cooperation and Development (OECD) iLibrary on Adverse Outcome Pathways.¹⁵⁷ With the help of information technology, existing knowledge can be pooled and shared. The OECD, the US Environmental Protection Agency, and the European Union Joint Research Centre are already collaborating on a shared resource “that covers the broad spectrum of biological pathways that are likely to be involved in human health and ecological risk assessment, the Adverse Outcome Pathway Knowledge Base”.¹⁵⁸

¹⁵⁴ Obviously, such modelling can also be used for the study of non-human animals.

¹⁵⁵ Marshall & Willett, 2018, p. 1952

¹⁵⁶ Marshall & Willett, 2018

¹⁵⁷ Bal-Price, et al., 2018

¹⁵⁸ Marshall & Willett, 2018, p. 1956



In-silico prediction methods for the evaluation of toxicity

SARs and QSARs

SARs and QSARs are mathematical models used for predicting biological activities of chemicals.

“A SAR is a qualitative relationships [sic] that relates a (sub)structure to the presence or absence of a property or activity of interest. The substructure may consist of adjacently bonded atoms, or an arrangement of non-bonded atoms that are collectively associated with the property or activity”.¹⁵⁹

For over a century, scientists have had some knowledge of the relationship between the structure of a substance and its toxicity. The knowledge of this structure-activity relationship (SAR) became well known, but was rarely gathered and systematically recorded until the 1980s. With progress in information technology, this changed:

“Since the early 1990s, the concept of applying compilations of structural alerts to evaluate and predict toxicity has progressed rapidly, with the expansion to other endpoints and effects. ... The development of compilations of structural alerts has also been stimulated by the availability of software that can capture them, and allow users to apply that knowledge. Bearing in mind that, until the late 1980s, the alerts were available only on paper, e.g. in journal articles or book chapters, they were difficult to use and open to misinterpretation, and may have been applied differently by different users”.¹⁶⁰

Quantitative structure-activity relationship (QSAR) modelling uses one or more quantitative parameters derived from a chemical structure to a property or activity of interest. The term “quantitative” in QSAR refers to the nature of the parameter(s) used

to make the prediction. Quantitative parameters enable the development of quantitative models. QSARs are mathematical models derived from a training set of example chemicals. “The training set includes the chemicals that were found to be positive and negative in a given toxicological study ... or to induce a continuous response ... that the model will predict”.¹⁶¹

The models can be used to predict a qualitative or quantitative endpoint.¹⁶² “It is assumed that chemicals that fit the same QSAR model may work through the same mechanism”.¹⁶³

QSAR models have been used, for example, for skin sensitisation, eye irritation and other human health hazards. They require a large database with information on the structure and toxicity of substances.

In 2007, changes in European legislation on chemical substances explicitly promoted the use of in-silico models, in particular (Q)SARs¹⁶⁴, as an alternative testing method to evaluate the safety of regulated substances:

“The legislation permitting the acceptance of in silico models to evaluate toxicity is Regulation EC 1907/2006, better known as Registration, Evaluation, Authorisation, and Restriction of Chemical substances (REACH). REACH specifies conditions for the use of in silico (Q)SAR models, and the European Chemicals Agency (ECHA) located in Helsinki, Finland implements REACH. ECHA offers guidance on how the (Q)SAR in silico method can help fulfill or support information requirements”.¹⁶⁵

So many different QSAR models are now available, that the proliferation of these models has been referred to as a “zoo of QSAR”¹⁶⁶, and this can make it difficult to choose the best one for the task at hand. Further, researchers

have pointed out that “many QSARs are difficult to interpret and cannot be used to define causal links. They represent correlation rather than causation”.¹⁶⁷

Progress in computer-based methods has seen improvements in dealing with incomplete data sets and making models less complicated:

“Recent advances in machine learning have resulted in models that can handle missing data and model multiple targets at once (multi-label learning, in case of toxicology for example multiple-hazard learning). These models can sometimes outperform single-label models by increasing the available data for training and by transferring concepts applicable to one label to predictions on another label. Multi-label models have the potential to simplify the QSAR space. Rather than having a model for every chemical property, a single model can predict many different chemical properties. In toxicology, many hazards are interrelated; thus, they can inform each others’ predictions. For example, a skin irritant is likely also an eye irritant, which means that information on both labels synergizes. So, the prediction of one hazard (label) informs other labels for the same and similar chemicals can improve predictions”.¹⁶⁸

For all in-silico predictions of toxicity, (Q)SAR and other models, standardised protocols need to be developed. “Such novel in silico toxicology (IST) protocols, when fully developed and implemented, will ensure in silico toxicological assessments are performed and evaluated in a consistent, reproducible, and well-documented manner across industries and regulatory bodies to support wider uptake and acceptance of the approaches”.¹⁶⁹

¹⁵⁹ European Chemicals Agency, 2008, p. 10

¹⁶⁰ Cronin & Yoon, 2018, p. 291

¹⁶¹ Myatt, et al., 2018, p. 6

¹⁶² European Chemicals Agency, 2008

¹⁶³ Raies & Bajic, 2016, p. 156

¹⁶⁴ SARs and QSARs are collectively referred to as (Q)SARs.

¹⁶⁵ Valerio, 2014, p. 1027

¹⁶⁶ Luechtefeld, Rowlands, & Hartung, 2018, p. 740

¹⁶⁷ Luechtefeld, Rowlands, et al., 2018, p. 739

¹⁶⁸ Luechtefeld, Rowlands, et al., 2018, p. 741

¹⁶⁹ Myatt, et al., 2018, p. 2

Read-across

Read-across is a method that is usually based on chemical structure information and uses QSAR approaches.¹⁷⁰ For example, the OECD QSAR Toolbox¹⁷¹ can be used to support read-across. The read-across method uses data from a substance for which toxicity information is available, to make predictions for a similar substance about which not much is known. In other words, a data-rich substance is used to make toxicity predictions for a structurally similar but data-poor substance.

While read-across is currently based on chemoinformatic¹⁷² approaches, it is not considered a QSAR: “although read-across is informed by chemical structure, it is strictly based on local similarity of a chemical with similar chemicals”.¹⁷³

“Read-across uses data on one or more analogs (the “source”) to make a prediction about a query compound or compounds (the “target”). Source compounds are identified that have a structurally or toxicologically meaningful relationship to the target compound, often underpinned by an understanding of a plausible biological mechanism shared between the source and target compounds. The toxicological experimental data from these source compounds can then be used to “read-across” to the specific target compound(s). Read-across is an intellectually-derived endpoint-specific method that provides justification for why a chemical is similar to another chemical (with respect to chemical reactivity, toxicokinetics, mechanism/mode of action, structure, physicochemical properties, and metabolic profile)”.¹⁷⁴

Read-across is fast and cost-effective, but it relies on subjective assessments of what constitutes a “similar” substance. When comparing chemical information for read-across, small changes in the structure of chemicals can result in big differences in the level of toxicity. This can lead to prediction errors. Adding other information, such as data from in-vitro tests, can improve the accuracy of the prediction.¹⁷⁵ Thus, knowledge of biological similarity enhances read-across. An international team of researchers offers the following outlook:

“The increasing availability of biological data via the data sharing depositories will augment such support of read-across and grouping by big data. The curation of such datasets and the respective data-sharing by companies, organizations and individual researchers needs to be further encouraged and possibly furthered with some incentives”.¹⁷⁶

In the EU, manufacturers and importers have to register information on chemical substances (that are produced or imported in volumes over one tonne a year) in a central database at the European Chemicals Agency (ECHA) in Helsinki. This is part of the REACH regulation system: registration, evaluation, authorisation and restriction of chemicals. ECHA’s 2017 report on the use of alternative methods revealed that the most common alternative method on analysing substances was read-across (63%), followed by weight of evidence (combining information from different sources), and QSAR predictions (34%)¹⁷⁷. Much of the information is still based on new or old experimental studies using animals, but ECHA reported that out of the 6,290



Read-across is a fast and cost-effective method for analysing chemicals.

substances analysed for the report, 89% had at least one data endpoint where an alternative to animal studies was used.

At present, regulatory agencies in the EU consider read-across the best method in the areas of skin and eye irritation.¹⁷⁸ “Read-across is an innovative approach that can be considered an alternative to animal testing – and at the moment it is probably the most effective method of reducing the use of lab animals”.¹⁷⁹ The authors of a review of in-silico methods for the prediction of chemical toxicity summarised the practical applications of the read-across method and provided examples of tools used:

“Read-across was applied to predict carcinogenicity, hepatotoxicity, aquatic toxicity, reproductive toxicity, skin sensitization, and environmental toxicity. Examples of tools implementing read-across are The OECD QSAR Toolbox, Toxmatch, ToxTree, AMBIT, AmbitDiscovery, AIM, DSSTox, or ChemIDplus”.¹⁸⁰

Read-across prediction is strengthened by the availability of high quality data, as well as agreed principles and guidance on how to group chemicals.¹⁸¹

¹⁷⁰ Zhu, et al., 2016

¹⁷¹ OECD, 2018c

¹⁷² Chemoinformatics is focused on extracting, processing and extrapolating meaningful data from chemical structures.

¹⁷³ Chesnut, et al., 2018, p. 414

¹⁷⁴ Myatt, et al., 2018, p. 7

¹⁷⁵ Chesnut, et al., 2018; Zhu, et al., 2016

¹⁷⁶ Zhu, et al., 2016, p. 178

¹⁷⁷ European Chemicals Agency, 2017b

¹⁷⁸ Archibald, et al., 2018

¹⁷⁹ Maertens, et al., 2016, p. 324

¹⁸⁰ Raies & Bajic, 2016, p. 152

¹⁸¹ Ball, et al., 2016; Patlewicz, et al., 2014

PBPK models

Physiologically-based pharmacokinetic¹⁸² (PBPK) models are “mathematical representations of the absorption, distribution, metabolism and elimination (ADME) of chemicals in humans or other animal species. They are used for multiple purposes, including the interpretation of in vitro toxicity data by in vitro to in vivo extrapolation (IVIVE) and the simulation of internal concentrations in the organism of interest”.¹⁸³

“Physiologically-based pharmacokinetic (PBPK) models are computational systems now commonly used in drug development and increasingly in regulatory toxicology. They predict the absorption, distribution, metabolism and excretion (ADME) of substances in the body, at different doses. PBPK models based on human rather than animal characteristics avoid the problem of species differences. They can also help predict variations in susceptibility between individuals and at different developmental life stages, which commonly occur but cannot be properly addressed by conventional animal testing. Functional PBPK models have developed alongside the rapid advances made in the use of human in vitro systems and in understanding gene function. It is now possible to predict ADME outcomes in ‘virtual humans’ with increasing confidence”.¹⁸⁴

In short, PBPK models are translational tools that can be used to link in-vitro and in-silico toxicity estimates to conditions in a living organism. Ready to use PBPK software tools are available.¹⁸⁵ PBPK models are also more generically referred to as PBK (physiologically based kinetic) models.¹⁸⁶

Expert systems

Expert systems are a varied group of models covering a combinations of SARs, QSARs and databases.¹⁸⁷ They derive toxicity predictions and estimates from a range of in-silico models. With access to large chemical and toxicological databases, increasing computational power, statistical algorithms for structure-activity modelling and powerful datamining tools, this area of research and testing has seen much progress in recent years and has become increasingly relevant and important for risk assessment required by government regulators.¹⁸⁸ In an overview of in-silico toxicology methods, expert systems were described as follows:

“Expert rule-based (or expert/ structural alerts). This methodology uses structural rules or alerts to make predictions for specific toxicological effects or mechanisms of toxicity. These rules are derived

from the literature or from an analysis of data sets generated by scientists. Structural alerts are defined as molecular substructures that can activate the toxicological effect or mechanism. ... The purpose of an in silico expert review is to evaluate the reliability of the prediction. The outcome of the review provides information to include in the assessment of the toxicological effect or mechanism. As part of this review, the expert might agree with, or refute, individual in silico predictions”.¹⁸⁹

¹⁸² Pharmacokinetics refers to the movement of drugs into, through and out of the body (drug absorption, distribution, metabolism and elimination).

¹⁸³ European Commission EU Science Hub, 2018a

¹⁸⁴ Langley, 2012, p. 24

¹⁸⁵ Cronin & Yoon, 2018

¹⁸⁶ for example, Paini, et al., 2019

¹⁸⁷ European Chemicals Agency, 2008

¹⁸⁸ Slikker, et al., 2018

¹⁸⁹ Myatt, et al., 2018, p. 6



In-silico tools for the evaluation of toxicity

RASAR

The REACH registration requirement has resulted in the ECHA database, a large database with information about thousands of chemicals. ECHA expected 60,000 registrations in 2018. This information is publicly available, but it is not presented in a standardised way that would make it easy to be read by a computer. A group of researchers has used natural language pattern matching to make the information machine-readable. They found, for example, that many chemicals have been repeatedly tested on animals:

“Interestingly, many chemicals have been tested more than once, some shockingly often: For example, one of the often challenged animal tests is the Draize rabbit eye test, where for more than 70 years now, test chemicals are administered into rabbit eyes. Two chemicals were tested more than 90 times, 69 chemicals were tested more than 45 times. ... Notably, the 9 most frequently done animal tests analyzed here consumed 57% of all animals for toxicological safety testing in Europe 2011”¹⁹⁰

After making the information machine-readable, they combined the ECHA database with several other large databases that are publicly available and developed algorithms that enable automatic read-across to model chemical properties. They named their innovation read-across structure activity relationships—RASAR.

With RASAR, the researchers mapped the relationships between chemical structures and toxic properties, based on 74 characteristics (such as whether a substance will cause eye irritation) to predict the properties of a substance. They found that this method can

predict toxic properties of any chemical substance more accurately than animal tests. Toxic substances were correctly predicted in 89% of cases. They estimated that by using the RASAR data fusion method, they would have saved 2.8 million animals and US \$490 million testing costs, and would have received more reliable data.

“It has recently been demonstrated that machine-learning software combined with big data can now be used to create sophisticated read-across-based tools that greatly outperform animal studies in predicting chemical safety, with an accuracy of 80%–95%, compared to 50%–70% for the respective animal tests”¹⁹¹

In addition to avoiding animal testing and obtaining superior results, RASAR can help avoid duplication of testing, achieves higher throughput, and is faster than conventional testing. But at this stage, it can't be used for complex human health effects, such as cancer. At the time of writing, RASAR research had just been published, and it is not yet clear whether regulators will accept this new testing method. Also, other researchers will carefully examine the claims made about RASAR.¹⁹² Thomas Hartung, one of the team that developed RASAR, commented that:

“In the future, a chemist could check RASAR before even synthesizing their next chemical to check whether the new structure will have problems. Or a product developer can pick alternatives to toxic substances to use in their products. This is a powerful technology, which is only starting to show all its potential”¹⁹³

OECD QSAR Toolbox

The OECD QSAR Toolbox¹⁹⁴ is a large, curated database developed by the European Chemicals Agency and the OECD. It is freely available online.¹⁹⁵ The OECD also provides training in the use of this resource, whose most important features are described as:

- “Identification of relevant structural characteristics and potential mechanism or mode of action of a target chemical.
- Identification of other chemicals that have the same structural characteristics and/or mechanism or mode of action.
- Use of existing experimental data to fill the data gap(s)”¹⁹⁶

REACHacross

Building on other in-silico methods, such as read-across and QSAR, and the availability of large databases, a group of scientists added machine-learning techniques. “A first implementation of machine learning-based predictions termed REACHacross achieved unprecedented sensitivities of >80% with specificities >70% in predicting the six most common acute and topical hazards covering about two thirds of the chemical universe”¹⁹⁷. However, this new tool has not yet been independently validated. The software tool is, however, available to help companies to meet their REACH requirements. As the REACHacross™ website notes,

“it predicts 8 required endpoints for REACH submissions. Users can produce Toxicology Assessment Reports for 6 key human health endpoints, including skin sensitization, acute dermal irritation, acute eye irritation, acute oral toxicity, acute dermal toxicity, mutagenicity and 2 key ecotoxicity endpoints of acute aquatic toxicity and chronic aquatic toxicity”¹⁹⁸

¹⁹⁰ Luechtefeld, Marsh, Rowlands, & Hartung, 2018, p. 199

¹⁹¹ Archibald, et al., 2018, p. 3

¹⁹² for example, Alves, et al., 2019

¹⁹³ Hartung, 2018

¹⁹⁴ OECD, 2018c

¹⁹⁵ Ford, 2016

¹⁹⁶ OECD, 2018c

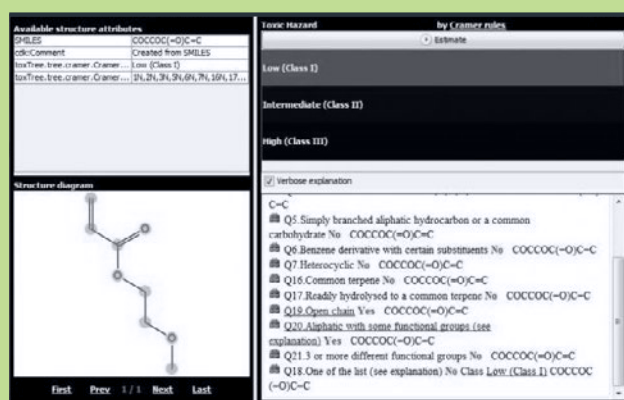
¹⁹⁷ Luechtefeld, Rowlands, et al., 2018, p. 732

¹⁹⁸ Underwriters Laboratories, no year

Toxtree

Toxtree is a free in-silico tool “that places chemicals into categories and predicts various kinds of toxic effects by applying decision tree approaches. The software is made freely available as a service to scientific researchers and anyone with an interest in the application of computer-based estimation methods in the assessment of chemical toxicity”.¹⁹⁹ The Joint Research Centre of the EU Commission commissioned the development of the software, and various contributors have since collaborated in its further development.

Toxtree tool



Source: European Commission EU Science Hub²⁰⁰

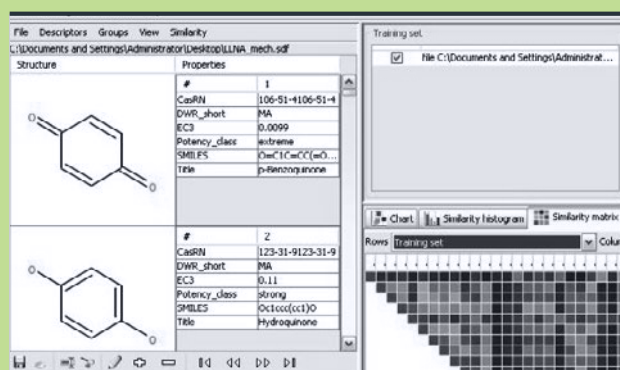
Toxmatch

“Toxmatch is an open-source software application that encodes several chemical similarity indices to facilitate the grouping of chemicals into categories and read-across.

The core functionalities include the ability to compare datasets based on various structural and descriptor-based similarity indices as well as the means to calculate pair wise similarity between compounds or aggregated similarity of a compound to a set.

The software is made freely available as a service to scientific researchers and anyone with an interest in the application of computer-based estimation methods in the assessment of chemical toxicity”.²⁰¹

Toxmatch tool



Source: European Commission EU Science Hub²⁰²

US EPA's GenRA tool helps predict whether a chemical is toxic

Current read-across relies on a subjective assessment of information about one drug to make predictions about another drug that is similar. Researchers at the US EPA have developed an automated read-across tool called Generalized Read-Across (GenRA) that aims to encode many expert assessments to make read-across a more systematic and data-based method of making predictions about the toxicity of specific drugs:

“In its current form, GenRA lets users find analogues, or chemicals that are similar to their target chemical, based on chemical structural similarity. The user can then select which analogues they want to carry forward into the GenRA prediction by exploring the consistency and concordance of the underlying experimental data for those analogues. Next, the tool predicts toxicity effects of specific repeated dose studies. Then, a plot with these outcomes is generated based on a similarity-weighted activity of the analogue chemicals the user selected. Finally, the user is presented with a data matrix view showing whether a chemical is predicted to be toxic (yes or no) for a chosen set of toxicity endpoints, with a quantitative measure of uncertainty”.²⁰³

This read-across resource is freely available on the EPA's website.

¹⁹⁹ European Commission EU Science Hub, 2016

²⁰⁰ European Commission EU Science Hub, 2016

²⁰¹ European Commission EU Science Hub, 2018b

²⁰² European Commission EU Science Hub, 2018b

²⁰³ United States Environmental Protection Agency, 2018a



Other in-silico approaches for the evaluation of toxicity

AOPs

An adverse Outcome Pathway (AOP) is a conceptual framework for risk assessment. It provides a biological explanation for a toxic event,²⁰⁴ and has been described in the following way:

“An AOP is a sequence of events that starts by a chemical effect at the molecular level (termed a Molecular Initiating Event) and progresses through changes (termed Key Events) in cells, tissues, and organs to produce an adverse effect in the body. AOPs act as a bridge between emerging methods of safety testing and, ultimately, what happens in the body in response to a particular substance”.²⁰⁵

AOPs are designed to enable targeted, fast, low-cost and tailored assessments. They can be continually improved, as new information is added to the pathways. “The eventual goal is to create a network, or web, of pathways that is sufficiently well described that the eventual effects of a chemical can be predicted with a limited spectrum of molecular information”.²⁰⁶

The OECD, which evaluates international chemical testing protocols, launched a program on the development and review of AOPs in 2002. The new AOPs are available on the AOP Wiki²⁰⁷, which allows scientists anywhere in the world to share, develop and discuss their knowledge of AOPs. Taking the comments on the AOP Wiki into account, the OECD publishes the endorsed AOPs on its website. These published AOPs can be updated on the Wiki.²⁰⁸ Effectopedia²⁰⁹, an online encyclopaedia of AOPs, is another example for the collaborative development and review of AOPs. The OECD’s Adverse Outcome Pathway Knowledge Base (AOP-KB) brings

the AOP Wiki, Effectopedia and two other AOP platforms together.²¹⁰ The following quote comments on the importance of AOP development:

“The safety evaluation of environmental and industrial chemicals is currently propagating the concept of adverse outcome pathways (AOPs). These developments have repercussions with drug development and safety assessments of drugs. Under the umbrella of Organisation for Economic Co-operation and Development (OECD) and closely linked to their chemical safety testing guideline program, AOPs are organizing in a crowdsourcing movement the mechanistic knowledge on how chemicals impact on human health and the environment. This happens, e.g. in the AOP-Wiki and for more quantitative AOPs in the Effectopedia platform. This is organizing existing knowledge on shared molecular initiating events and the subsequent key events along the chain from cellular to tissue, organ, organism, and population effects”.²¹¹

IATA

Integrated Approaches to Testing and Assessment (IATA) are approaches for making decisions about the toxicity of substances that are based on multiple information sources, such as physicochemical properties, non-testing methods such as QSAR models and read-across, and testing methods such as in-vitro and in-vivo. The identification of an AOP is a key component of this approach.²¹² IATA approaches are mainly used for regulatory purposes. IATA work and are used in the following ways:

“Integrated Approaches to Testing and Assessment (IATA) are a flexible tool for chemical safety assessment, based on the integration and translation of data derived from multiple methods and sources. In addition to traditional in vitro and in vivo tests, IATA are increasingly incorporating new approach methods, such as high-throughput screening and high-content imaging methods, along with computational approaches that are used as a means of data generation, interpretation and integration”.²¹³

“Models are no longer applied in isolation to determine chemical safety; there has been a growing global trend towards the development and use of multiple strands of information within Integrated Approaches to Testing and Assessment (IATA) for safety assessment. Nearly all IATA involve the use of existing data, (Q)SAR predictions and/or read-across that are amenable to being integrated into computational workflows”.²¹⁴

As this approach includes subjective expert judgement (weight-of-evidence), it is not easy to standardise across industry sectors and countries.

However, IATA frameworks for skin irritation, skin corrosion, eye irritation, serious eye damage and skin sensitisation have already been adopted internationally.²¹⁵

²⁰⁴ Taylor, 2019

²⁰⁵ Ram, 2019, p. 367

²⁰⁶ Marshall & Willett, 2018, p. 1956

²⁰⁷ AOP-Wiki team, no year

²⁰⁸ OECD, 2018a

²⁰⁹ OECD, 2016

²¹⁰ OECD, 2019

²¹¹ Hartung, 2017b, p. 1

²¹² Clippinger, et al., 2018

²¹³ Worth & Blaauboer, 2018, p. 301

²¹⁴ Cronin, Madden, Yang, & Worth, 2019, p. 40

²¹⁵ Casati, 2018; Zuang, et al., 2017



Computer modelling of health and disease

Computer models are also used to simulate (virtual) organs or the human body, and to explore various aspects of diseases. Computer models can link many processes together, something which is not possible to achieve with animal models. For example, atherosclerosis is a common cardiovascular disease that is caused by a combination of factors and can be studied with the help of computer modelling:

“As a disease that depends on multiple factors operating on different length scales, the natural framework to apply to atherosclerosis is mathematical and computational modelling. A computational model provides an integrated description of the disease and serves as an *in silico* experimental system from which we can learn about the disease and develop therapeutic hypotheses”.²¹⁶

Computer modelling can integrate electrical and mechanical processes into electromechanical models. These models are useful to study, for example, implanted cardiac devices such as pacemakers.²¹⁷ Modelling of the respiratory system is another area of study.²¹⁸ Such models can be created for individual patients. Computer modelling makes an important contribution to the discovery of new knowledge. Below, a team of experts in anaesthesiology²¹⁹ comment on this contribution from their perspective:

“... complex *in silico* models have been applied to pathophysiological problems to provide information which cannot be obtained practically or ethically by traditional clinical research methods. These experiments have led to the development of significant insights in subject matters ranging from pure physiology to congenital heart surgery, obstetric anaesthesia

airway management, mechanical ventilation and cardiopulmonary bypass/ventricular support devices”.²²⁰

Other applications of computer modelling include virtual reality and surgical simulation. These will be covered in the section on simulators.

Computer modelling in planning and performing surgery in children and young adults with congenital heart disease

Congenital heart disease (CHD) is the most common form of birth defect in the US and Canada. Nearly all forms of CHD lead to long-term complications, and the cardiovascular autonomy of patients varies. Hence, there is great interest and potential in the application of biomedical engineering (BME)-based modelling and simulations to predict the outcomes of surgical and other interventions.

For example, advanced imaging and 3D printing technology allows the surgeon to print a 3D model of an individual patient's heart and then perform surgery on the printed model. Computational fluid dynamics (CFD) involves numerical analysis to solve problems that involve fluid flows, such as blood flow.

“Application of advanced CFD analyses on a patient's preoperative anatomy and on the proposed postoperative solution allows a higher level of confidence that the intended approach will yield the desired result”.²²¹ A medical doctor reported his observations regarding the opportunities of computer modelling for his work as follows:

“Despite the intensity of the work involved, surgical planning with computational modelling offers unique insights and tremendous potential for the care of patients with CHD. Several centres have dedicated laboratories working in this field, and a few multicentre collaboratives have formed. Congenital cardiology is moving into an era of ‘personalized medicine,’ and surgical planning with computational modelling and CFD offers hope that each patient's interventional procedure can be planned and analyzed preoperatively on the basis of their patient-specific characteristics to ensure optimal long-term outcomes”.²²²

²¹⁶ Parton, McGilligan, O’Kane, Baldrick, & Watterson, 2016, p. 562

²¹⁷ Pluijmert, et al., 2015

²¹⁸ Clark, Kumar, & Burrowes, 2017

²¹⁹ A medical speciality that encompasses anaesthesia, intensive care medicine, critical emergency medicine, and pain medicine

²²⁰ Colquitt, Colquhoun, & Thiele, 2011, p. 499

²²¹ Slesnick, 2017, p. 1160

²²² Slesnick, 2017, p. 1168

Computational psychiatry

“Translating advances in neuroscience into benefits for patients with mental illness presents enormous challenges because it involves both the most complex organ, the brain, and its interaction with a similarly complex environment. Dealing with such complexities demands powerful techniques. Computational psychiatry combines multiple levels and types of computation with multiple types of data in an effort to improve understanding, prediction and treatment of mental illness”.²²³

Computational psychiatry, similar to other computational predictive approaches, includes two methods: data-driven analysis uses methods from machine learning, such as statistics, to improve classification of disease, predict treatment outcomes or improve treatment selection; and theory-driven approaches “mathematically specify mechanistically interpretable relations between variables (often including both observable variables and postulated, theoretically meaningful hidden variables)”.²²⁴ Unlike data-driven approaches, theory-driven methods incorporate prior knowledge or hypotheses. Both approaches can be combined. Some of the advantages and limitations of computational psychiatry are noted below:

“Data-driven approaches have started to bear some fruit for clinically relevant problems, such as improving classification, predicting treatment response and aiding treatment selection. These approaches, however, are limited in their ability to capture the complexities of interacting variables in and across multiple levels. Theory-driven modeling efforts, on the other hand, have yielded key insights at many levels of analysis concerning the processes underlying specific disorders, but for the most part have yet to be applied to clinical problems”.²²⁵

In-silico drug trials can be more accurate than animal testing

A group of computer scientists at the University of Oxford have demonstrated that in-silico drug trials are more accurate than animal tests at predicting clinical pro-arrhythmic cardiotoxicity, where a drug causes an irregular heart beat or stops the heart. The researchers tested 62 compounds at multiple concentrations and found accuracy rates of 89-96%, compared to 75-85% accuracy from animal testing. Different species of animals, such as rats, mice, rabbits, guinea pigs, dogs and pigs, are still used in drug development to predict side effects for the human heart.²²⁶

RepoTrial

Developing a new drug takes 10 to 15 years and is very expensive. Drug repurposing, that is finding a different use for an already approved drug, is faster, costs less, and requires fewer or no animal tests. One reason for this is that potential side effects of the drug are already known.

The RepoTrial project uses computer-based algorithms to find out whether already registered drugs may work for other diseases that have some similar characteristics, but may relate to different organs or body parts. The drugs will be tested on virtual patients, and finally on real patients.²²⁷

²²³ Huys, Maia, & Frank, 2016, p. 404

²²⁴ Huys, et al., 2016, p. 404

²²⁵ Huys, et al., 2016, p. 411

²²⁶ Passini, et al., 2017; Passini, Rodriguez, & Benito, 2018

²²⁷ RepoTrial, 2018

Machine learning can predict individual cancer patients' responses to chemotherapy drugs

Researchers at Georgia Institute of Technology in the US have developed algorithms to predict the response of individual cancer patients to chemotherapy drugs. They used their algorithms to predict the responses of 152 individual cancer patients to chemotherapeutic drugs, based on gene expression profiles of each individual's tumour, with an accuracy of over 80%.²²⁸ The new tool is available to clinicians at no cost and can help them choose the chemotherapy drug best suited for individual patients.

“Accurate predictions in cancer biology, as in all areas of science, can be based upon established cause-and-effect relationships or upon significant correlations detected in large sets of relevant data. While we are well on our way to the day when we may fully understand the molecular causes of all cancers and treat them accordingly, we are not there yet. One promising interim solution is the application of prediction algorithms derived from ML-detected correlations between the molecular profiles of large numbers of cancers and associated responses to a variety of therapeutic drugs”.²²⁹

The Virtual Physiological Human project

In 2005, a small group of researchers and officers from the European Commission started the Virtual Physiological Human (VPH) project which a few years later resulted in the establishment of the Virtual Physiological Human Institute for Integrative Biomedical Research (VPH Institute). In 2011, the VPH Institute announced its three targets:

“1. Digital patient. The VPH for the doctor; patient-specific modeling to support medical decisions.

2. In silico clinical trials. The VPH for the biomedical industry; collections of patient-specific models to augment the preclinical and clinical assessment of new biomedical products; in silico technologies for the reduction, refinement, and partial replacement of animal and human experimentation.

3. Personal health forecasting. The VPH for the patient/citizen; subject-specific real-time simulations, based on data collected by wearable and environmental sensors, that provide advice to individuals affected by conditions requiring careful self-management or to people simply at risk of developing certain diseases”.²³⁰

The VPH Institute is an international non-profit organisation whose collaborators envisage that VPH models will enable predictive and personalised medicine. Personalised predictions will support and improve clinical decisions and help with personalised prevention.²³¹

²²⁸ C. Huang, et al., 2018

²²⁹ C. Huang, et al., 2018, p. 5

²³⁰ Viceconti & Hunter, 2016, p. 107

²³¹ Viceconti & Hunter, 2016

Studies with human volunteers

Studies with human volunteers are not new. Examinations of cadavers have been conducted for centuries, though with modern technologies the methods have become more sophisticated. Population-based studies appeared first during the 19th century, and microdosing goes back several decades.



Post-mortem studies

Donated tissues after the death of a person can be studied to gain insight into cell-level changes in human illnesses. For example, the brains of stroke victims can be studied after death²³² and cadavers can be used in training surgical skills.²³³ Such studies can replace studies on living non-human primates and other animals.

Animal models are also used in behavioural tests, such as the despair test – where rats or mice are forced to swim until they are exhausted and stop swimming, then antidepressants are administered to see if this prolongs the time they are able to swim – to study stress, anxiety, depression, and obsessive-compulsive behaviours. Researchers who conduct these tests assume that the animals accurately mimic human psychiatric conditions. However, the human brain's architecture and physiology is much more complex than that of rodents or monkeys.²³⁴

“Diagnosing ‘depression’ in a monkey is at odds with the successful ongoing process of clarifying psychiatric diagnoses by using DSM criteria. It is not possible to ascertain feelings of worthlessness and excessive guilt, indecisiveness, and thoughts of death from observations of monkeys”.^{235 236}

Post-mortem brain studies are useful to gain more knowledge about psychiatric illnesses, in particular in combination with the approaches of genomics and proteomics. For example, post-mortem tissue from the prefrontal cortex of people who lived with schizophrenia have led to new insights about this disease.²³⁷ Some advantages of studying post-mortem tissues are reported below:

“Indeed, one of the major limitations to our knowledge of human neurological diseases resides partly in the limits inherent to animal models, which mimic some aspects of the human neurological disorder without reproducing its complexity arising from both genetic and environmental factors. For example, more than 50 different animal models have been generated to explore Alzheimer’s disease (AD) and more than 20 models are available for the study of schizophrenia without clear consensus about the similarities with human disease. The underuse of post-mortem human brain tissue also impedes the deeper understanding of the pathophysiological processes ongoing in the diseased brain”.²³⁸

Brain tissues available for research are kept in brain banks where they are processed (for example, frozen) by standard procedures. Most brain banks keep tissues donated by individuals or their families, but very few have specimens from longitudinal cohort studies.^{239 240}

²³² Gordon & Langley, 2008

²³³ Thomson, Poudrier, Stranix, Motosko, & Hazen, 2018

²³⁴ Menache, 2012

²³⁵ Menache, 2012, p. 5

²³⁶ Diagnostic and Statistical Manual of Mental Disorders (DSM). It defines and classifies mental disorders for the purposes of diagnosis, treatment and research.

²³⁷ Lewis, 2002

²³⁸ Gomez-Nicola & Boche, 2015, p. 1

²³⁹ Repeated observation of groups of people over long periods of time.

²⁴⁰ Paraizo Leite & Tenenholz Grinberg, 2015



Population-based studies

Epidemiology is the study of diseases and other health-related states in groups (populations) of people, in particular how, when and where they occur. Epidemiologists want to discover what factors are associated with diseases (risk factors), and what factors may protect people against disease (protective factors). Epidemiological studies discover the correlation between certain behaviours (such as smoking), demographic factors (such as age, sex), constitutional factors (such as blood group or immune status) or circumstances (such as living in an area with high pollution) and health-related conditions.

A population or group can be studied in different ways, for example, by questionnaire, by taking measurements (such as blood pressure), by analysing blood specimens, or by examining healthcare records. Epidemiologists use statistical methods to conclude whether the differences they find are real or due to chance.

Common types of epidemiological studies include:

- **Cohort studies** – observational studies used by epidemiologists looking into the factors that affect the health and illness of populations. Longitudinal studies are a form of cohort studies, which follow groups of people over time. They can be prospective or retrospective. The cohort study design is a good scientific method for measuring the effects of a suspected risk factor.

- **Case control studies** – observational studies comparing patients who have a disease or outcome of interest (cases) with patients who do not have the disease or outcome (controls). Also, looking back retrospectively to compare how frequently the exposure to a risk factor is present in each group to find out about the relationship between the risk factor and the disease.
- **Cross-sectional studies** – observational studies of a distinct population at a single point in time or over a short period. Exposure to the risk factor(s) and outcome are determined at the same time.

More recently, omics technologies have been used in epidemiological research:

“-Omics technologies have substantially transformed epidemiological research and advanced the paradigm of molecular epidemiology, which focuses on underlying biology (pathogenesis) rather than on empirical observations alone. ... The -omics technologies that have been applied in epidemiological research, however, have now expanded beyond genomics to include epigenomics, proteomics, transcriptomics, and metabolomics”²⁴¹

Biobanks can become an important resource for such research.

Epidemiological studies can replace animal studies. For example, if researchers want to find out whether a high carbohydrate or a high protein diet is better for weight loss, this can be studied in populations that already follow these diets. Similarly, forcing mice into small tubes and making them inhale tobacco smoke²⁴² is not necessary as there are still many human smokers who could be asked to participate in an epidemiological study.

Many nutrition and diet studies use mice or rats. The digestive systems of these animals are very different to those of humans. Some researchers even acknowledge that the knowledge gained from studies with animals can't be applied to humans. For example, a research group at the University of Copenhagen found a way to stop mice gaining weight despite being fed a diet high in fat. They discovered that mice who were lacking the enzyme NAMPT would not put on more weight on a high-fat diet than on a healthy diet. However, decreasing this enzyme in obese humans is not an option, because NAMPT is essential for healthy muscle function.²⁴³

²⁴¹ National Academies of Sciences, 2017, p. 4

²⁴² Hsu, et al., 2015

²⁴³ Nielsen, et al., 2018



Microdosing

The concept of microdosing goes back to the late 1990s and is intended to investigate basic drug properties. In drug development, a microdose is a dose of a compound (chemical elements of more than one element) that is intended to be subpharmacologic²⁴⁴ when administered. It is 1/100th of the known or expected active dose or 100 µg²⁴⁵/adult, whichever is the smaller.²⁴⁶

The two assumptions behind microdosing are that the best model for humans are humans, and that the pharmacokinetics²⁴⁷ observed after microdosing is an acceptably accurate predictor of therapeutic doses of a compound.²⁴⁸ Also referred to as Phase 0 of clinical trials, microdosing is usually employed at the very start of clinical tests with humans. It can provide useful information to help decide whether the new compound or drug should be developed further, and whether it may be safe to progress to further human testing. High failure rates in the clinical phase of drug development are now at 95%: “This illustrates the limited predictivity of our predominant tool – the animal model”.²⁴⁹ Microdosing could screen out drugs destined to fail earlier, faster and cheaper. Further, unlike animal testing, it can provide vital information about different groups of patients:

“Microdosing is an attractive approach for the study of new and existing drugs in vulnerable populations (children, pregnant women, elderly, hepatically and renally impaired), who are routinely excluded from clinical trials due to safety concerns”.²⁵⁰

Microdosing can replace the use of animals in safety testing of new compounds and drugs. Mostly but not always, these animals are rodents. According to international guidelines, a single dose given to a mouse or rat is “1000-fold the human dose, followed by 2 weeks of observation”.²⁵¹

Non-human primates are also used for animal testing in drug development. In the US, cynomolgus macaques (crab-eating macaques) are the most commonly used type of monkeys used for this purpose.²⁵²

²⁴⁴ A subpharmacological dose is a minute, safe dose of a test compound.

²⁴⁵ A µg is a microgram.

²⁴⁶ Rowland, 2012

²⁴⁷ Pharmacokinetics is the study of the time duration of drug absorption, distribution, metabolism and excretion.

²⁴⁸ Rowland, 2012

²⁴⁹ Hartung, 2017a, p. 26

²⁵⁰ Lappin, Noveck, & Burt, 2013, p. 818

²⁵¹ Svendsen, et al., 2016

²⁵² Feister, DiPietrantonio, Yuenger, Ireland, & Rao, 2018

Simulators

The availability and use of simulators in medical and veterinary training is increasing. Simulators are also replacing live animals in primary and secondary schools. Training simulators are either virtual reality (VR)-based or physical model (PM)-based. Apart from replacing live animals in education and training, VR simulators have great potential for training people in remote locations, for example, training students and surgeons in developing countries.

Virtual reality-based systems can be grouped in immersive VR, non-immersive VR, augmented reality (AR) and mixed reality. In immersive VR, the real world is completely blocked from view and users experience that they are immersed in that virtual world. Non-immersive VR blocks the real world, but users remain aware that they are viewing a virtual environment. The user's view is commonly blocked by a head-mounted display (HMD). This is how it works:

“In the immersive and non-immersive VR systems, the user wears an HMD that occludes the real world, and the user can maneuver within the virtual environment through movements of his or her head and by physically walking around. Motion sensors in the HMD track head movements, while external cameras track the user as they walk. These movements are then translated into motion within the virtual world. Alternatively, the user can maneuver through the virtual environment and manipulate objects using a handheld device with haptic feedback that give the illusion of actually interacting with the virtual environment and objects within it”²⁵³

In AR, the real world is not blocked from the user's view. Virtual objects are superimposed, and users are able to interact at the same time with the real world and the virtual objects. The virtual objects are transparent in the real world in daylight, similar to a hologram. In mixed reality, the virtual images appear solid. In AR and mixed reality, users wear glasses that do not block out the real world.²⁵⁴

The beginnings of VR and AR go back to the 1960s. The US Air Force and NASA were early adopters with flight simulators designed to train fighter pilots introduced during the 1990s²⁵⁵ and head-mounted displays (HMDs) used for astronauts. By 2010, computer technology was advanced enough so that truly immersive VR and AR systems could be developed.²⁵⁶

AR is now widely used in education from kindergarten to high school and can be used with computers or mobile devices, without head-mounted displays.²⁵⁷

VR and AR systems have great potential for the training of surgical skills and, for example, replace the use of rat vessels that are now used in microsurgery training.²⁵⁸ VR and AR avoid the need for animals or cadavers and provide a risk-free environment for practicing

surgical skills and decision making.²⁵⁹ “Ultimately, this would lead to greater efficiency, improved patient care, and minimization of technical errors that are inherent to the surgical learning curve”.²⁶⁰ Nevertheless, virtual reality systems are not yet able to fully simulate the tactile experience of the clinical situation with a real patient.²⁶¹

Physical model-based simulators are available from companies that specialise in their development and production, or can be “home-made” by researchers. A well-known example of a commercially produced simulator is TraumaMan.²⁶²

The American College of Surgeons' (ACS) Advanced Trauma Life Support (ATLS) program has become the accepted standard of care for the initial assessment and treatment of trauma patients. Since 1976, the ATLS program has been training health professionals and is now offered in 86 countries. Initially, surgical skills were practised on the chests, throats, abdomens and limbs of live dogs, pigs, sheep and goats. Using these animals is costly and the animal models can't properly simulate the clinical conditions of human emergency situations. Also, public disapproval of animal models has increased. So in 2001 the ACS

²⁵³ Pelargos, et al., 2017, p. 2

²⁵⁴ Pelargos, et al., 2017

²⁵⁵ Akçayır & Akçayır, 2017

²⁵⁶ Pelargos, et al., 2017

²⁵⁷ Akçayır & Akçayır, 2017

²⁵⁸ Thomson, et al., 2018

²⁵⁹ Vaughan, Dubey, Wainwright, & Middleton, 2016

²⁶⁰ Pelargos, et al., 2017, p. 2

²⁶¹ Thomson, et al., 2018

²⁶² Simulab, 2017

approved the use of human cadavers and the TraumaMan system to replace live animals in trauma training. TraumaMan is a surgical simulation manikin with lifelike skin, tissue, internal organs and bones. It bleeds when cut, and simulates breathing using a ventilator. The ACS has since approved other simulation systems. Nearly all ATLS programs in the US and Canada have now replaced animal use with simulation systems.²⁶³

A wide range of simulators is available and well suited for education, training and professional development:

“Immersive, highly visual and often 3D, virtual reality simulators closely replicate real-life with incorporation of physical interfaces and haptic feedback. Virtual reality simulators are technically advanced, and available in a range of sizes and shapes. Some incorporate gaming industry headsets and cellular phones, while others involve large-scale simulators resembling carnival rides. The field of virtual reality training in trauma is growing exponentially. Downloadable apps are currently being developed that, with the addition of a \$20 headset, can provide an immersive trauma scenario experience”.²⁶⁴

A review of studies comparing animal use in veterinary education and training with non-animal methods found that student learning outcomes were at least as good with humane teaching methods, in some cases even better. Non-animal methods also had other benefits, such as:

“time and cost savings, enhanced potential for customisation and repeatability of the learning exercise, increased student confidence and satisfaction, increased compliance with animal use legislation, elimination of objections to the use of purpose-killed animals, and integration of clinical perspectives and ethics early in the curriculum. The evidence demonstrates that veterinary educators can best serve their students and animals, while minimising financial and time burdens, by introducing well-designed teaching methods not reliant on harmful animal use”.²⁶⁵

This review was published more than ten years ago, and since then many more humane teaching tools have become available.

Similarly, a review of VR-based simulators for spine surgery found that the VR training was more effective than traditional approaches. In particular, VR training was very effective in teaching surgical technical skills and knowledge.²⁶⁶ Compared to the use of live animals, simulators have the advantage that students can repeat practising their surgical skills until they are competent.

There are also various online data bases and digital representations of human and animal bodies and body parts. For example, BioDigital²⁶⁷ claims to be “The World’s First Human Visualization Platform”, and the SPM Anatomy toolbox²⁶⁸ offers maps of the human brain and functional imaging data.

²⁶³ Gala & Crandall, 2018

²⁶⁴ Quick, 2018, p. 448

²⁶⁵ Knight, 2007, p. 91

²⁶⁶ Pfandler, Lazarovici, Stefan, Wucherer, & Weigl, 2017

²⁶⁷ BioDigital, 2018

²⁶⁸ Institut für Neurowissenschaften und Medizin, 2017

San Antonio Military Medical Center's simulation center



Source: *Airman Magazine*²⁶⁹

A simulator for a range of human functions



A simulator that can breathe, bleed, vomit and respond physiologically to treatment given such as medications and give feedback to procedures such as defibrillation.

Source: *Laviv Yaacov, Sheba Medical Center, Israel*²⁷⁰

Froggipedia

Froggipedia²⁷¹ is a virtual dissection app and Apple's top iPad app of 2018. It lets users dissect 3D frogs and is a great learning tool for the classroom. No frogs are harmed.

²⁶⁹ Airman Magazine, no year

²⁷⁰ Yaacov, 2015

²⁷¹ Designmate (I) Pvt. Ltd., 2019

Human-relevant research and testing methods/technologies and their application

Methods/ technologies that can replace animals in biomedical research and testing	Chemicals/ drug testing for toxicity & effectiveness	Regenerative medicine & other treatments	Personalised/ precision medicine	Organ/ disease studies	Large scale & fast studies/ tests	Education & training	Provides access to human tissues
In-vitro methods							
Organoids, organs-on- chips	✓	✓	✓	✓	✓		
Biobanking							✓
Omics technologies			✓	✓	✓		
Stem cell technologies	✓	✓	✓	✓			
3D and 4D bioprinting	✓	✓		✓	✓		
Robotic testing	✓			✓	✓		
In-silico methods							
Prediction methods (SARs & QSARs, Read- across, PBPK models, Expert systems) and tools	✓			✓	✓		
AOPs, IATA	✓			✓	✓		
Computer modelling	✓		✓	✓		✓	
Studies with human volunteers							
Post-mortem studies				✓		✓	✓
Population- based studies	✓			✓			
Microdosing	✓						
Simulators							
Virtual reality & physical model-based simulators			✓	✓		✓	

Efforts by governments and the scientific community to replace animal experimentation

New alternatives to animal research and testing can only be developed with the support and collaboration of governments, the scientific community and industry. Philanthropic organisations also provide financial support. In the following, examples of major initiatives by governments, the scientific community and other stakeholders are listed.



Policies and collaborations

In particular in the European Union and the US, governments have issued policies that aim to replace, reduce and refine the use of animals in biomedical research and testing. Government regulators, researchers in academia and industry and other interested parties are collaborating to implement these policies. Examples of major policies and collaborations include the following:

United States

- **A strategic roadmap for establishing new approaches to evaluate the safety of chemicals and medical products in the United States**

“This strategic roadmap is a resource to guide U.S. federal agencies and stakeholders seeking to adopt new approaches to safety and risk assessment of chemicals and medical products that improve human relevance and replace or reduce the use of animals. This document was developed with input from members of 16 federal agencies, multiple interagency workgroups, and input from the public. As such, it represents a consensus perspective that does not necessarily reflect opinions

or policy of any specific agency or workgroup, and should not be taken as a commitment by any federal agency”.²⁷²

- **Toxicology in the 21st Century**

“Toxicology in the 21st Century (Tox21) is a US federal research collaboration that is developing alternative, non-animal methods to quickly and efficiently test thousands of chemicals for potential health effects. These approaches use advances in robotics technology to test chemicals for their potential to disrupt processes in the human body, which may lead to negative health effects”.²⁷³

²⁷² Interagency Coordinating Committee on the Validation of Alternative Methods, 2018

²⁷³ U.S. Environmental Protection Agency, 2017, p. 1

European Union

- **FDA's Predictive Toxicology Roadmap**

In response to the FDA Commissioner's request for a roadmap for integrating predictive toxicology methods into safety and risk assessments, the FDA's Toxicology Working Group released the Predictive Toxicology Roadmap in 2017.²⁷⁴

"The Predictive Toxicology Roadmap of the FDA, which aims to integrate new approaches while reducing animal tests, lists the following human-based methods as especially promising new technologies for use in predictive toxicology: microphysiological systems; alternative test methods for reproductive toxicity testing; computational toxicology; in vitro alternatives; and read-across methodologies. Although evaluation of these methods is crucial to understanding their value in predicting human in vivo outcomes, human-based approaches can be expected to provide data that are more relevant than animal-based methods because they enable scientists to investigate how drugs interact with human cells, tissues, and biological processes directly, removing the need for cross-species extrapolation".²⁷⁵

- **Directive 2010/63/EU on the protection of animals used for scientific purposes**

In 2010, the European Parliament and the Council of the European Union adopted Directive 2010/63/EU on the protection of animals used for scientific purposes, to come into effect on 1 January 2013, replacing the previous 1986 directive. All use of live animals for scientific and educational purposes must be carried out in compliance with this Directive. 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".²⁷⁶

- **Ban on animal testing of cosmetics**

"The EU Cosmetics Regulation prohibits animal testing of finished products since 2004 and of cosmetic ingredients since 2009, reinforced by a marketing ban of cosmetics finished products tested on animals since 2004 and for cosmetics containing ingredients tested on animals since 2013".²⁷⁷

- **European Partnership for Alternative Approaches to Animal Testing**

"The European Partnership for Alternative Approaches to Animal Testing (EPAA) is an unprecedented voluntary collaboration between the European Commission, European trade associations, and companies from 7 industry sectors. The partners are committed to pooling knowledge and resources to accelerate the

development, validation and acceptance of alternative approaches to animal use in regulatory testing. The overall aim is the replacement, reduction and refinement (3Rs) of animal use in regulatory testing".²⁷⁸

- **Transition to non-animal research in the Netherlands**

- The Dutch Government aims to be a world leader in alternatives to animal research by 2025. In 2016, the Netherlands National Committee for the protection of animals used for scientific purposes presented a report to the Minister of Agriculture with timelines for phasing out animal research. As a first step, by 2025 animal procedures will be phased out in regulatory safety research (such as testing of chemical substances, food ingredients, pesticides, medicines and vaccines). It will take longer to transition to animal-free research in the fields of fundamental scientific research and applied and translational research.²⁷⁹

- **Innovate UK. A non-animal technologies roadmap for the UK**

"This roadmap, vision and strategy for non-animal technologies in the UK has been drawn up by Innovate UK, the National Centre for the Replacement Refinement and Reduction of Animals in Research, the Biotechnology and Biological Sciences Research Council, the Defence, Science and Technology Laboratory, the Engineering and Physical Sciences Research Council and the Medical Research Council, and has been published on their behalf by Innovate UK. It is intended to guide the efforts of all those

²⁷⁴ U.S. Food and Drug Administration, 2017

²⁷⁵ Baker, et al., 2018, p. 625

²⁷⁶ European Parliament and the Council of the European Union, 2010, p. 2

²⁷⁷ Dal Negro, et al., 2018, p. 33

²⁷⁸ European Commission, 2018a

²⁷⁹ Netherlands National Committee for the protection of animals used for scientific purposes, 2016

working in this area. The issues outlined and the recommendations have come out of extensive discussions between the six organisations that are endorsing the roadmap and with many other key stakeholders. The participation and endorsement by the six organisations reflects their continuing interest in non-animal technologies, but should not be construed as a commitment to ensuring its delivery”.²⁸⁰

- **ORCHID (organ-on-chip in development)**

The ORCHID project is a collaboration between seven research institutions from six European countries. The project’s main goal is to create a roadmap for organ-on-chip technology and to build a network of all relevant stakeholders.²⁸¹

- **EU-ToxRisk**

This is a major collaborative six-year project. It was launched in 2016 with a budget of €30 million, funded by the EU. The project “aims to make progress towards animal-free safety assessments and tackles complex areas of toxicology, such as repeated-dose and reproductive toxicity”.²⁸²

- **EuroMix**

EuroMix (European Test and Risk Assessment Strategies for Mixtures) is another collaborative project. The project examines how a mixture of multiple chemicals affect human health – chemicals that may be part of the food we eat, may be inhaled, or may come into contact with our skin. The project uses computer-based methods and in-vitro assays.²⁸³

International

- **PETA International Science Consortium**

“The Science Consortium and its members promote the implementation of animal-free testing approaches through multiple efforts, including:

- providing financial support toward the development and validation of non-animal test methods
- organising expert working groups to tackle the development of new approaches to address regulatory requirements when non-animal methods do not exist or require further optimisation
- providing technical support to companies and researchers seeking to replace, reduce, or refine the use of animal tests
- publishing manuscripts, developing technical analyses, presenting at international scientific conferences, and hosting free webinars to ensure that information regarding the use of non-animal methods is accessible to all audiences
- interacting with national and international regulatory bodies and standards organisations to ensure that opportunities exist to increase and harmonise the use of validated non-animal test methods. This includes attending Organisation for Economic Co-operation and Development (OECD) meetings through the International Council on Animal Protection in OECD Programmes (ICAPO) to ensure the best possible science and widest possible integration of alternatives to in vivo test methods in OECD guidelines”.²⁸⁴

- **BioMed21** (biomedical research for the 21st century)

“The BioMed21 Collaboration (<https://www.biomed21.org>) grew out of a 2015 review paper authored by a diverse group of stakeholders representing civil society, research funding, academic, regulatory, corporate and other communities, which recognized the human relevance and translational limitations of the conventional paradigm in biomedical research and drug discovery and the need for change”.²⁸⁵

- **OECD Integrated Approaches to Testing and Assessment (IATA)**

The OECD provides guidance documents for IATA and facilitates the sharing of case studies that “explore the use of novel methodologies in Integrated Approaches to Testing and Assessment within a regulatory context”.²⁸⁶ New approaches are needed to assess the many chemicals in use today that have never been tested. Testing these chemicals in the conventional way would increase the use of animals and be too costly and slow.

²⁸⁰ Innovate Uk, 2015, p. 2

²⁸¹ Mastrangeli, et al., 2018

²⁸² European Commission, 2018c, p. 6

²⁸³ National Institute for Public Health and the Environment, no year

²⁸⁴ PETA, 2019

²⁸⁵ Triunfol, et al., 2018, p. 1230

²⁸⁶ OECD, 2018b



Validation

Any new drug testing method has to be checked to make sure it is safe and effective. Governments regulate drugs and other substances. They have to be convinced that the new methods are as good or better than conventional ones. The process of checking whether the claims about new methods are accurate is called validation. It is a key step towards the acceptance of a test method by the regulators. Several countries have validation centres that assess new methods and technologies. The first validation centre, the European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) was established in 1991, and since then similar centres have been set up in the US (1995), Japan (2005), South Korea (2010), Brazil (2013) and Canada (2017).

These validation centres collaborate in streamlining and standardising the validation process. Together with the Chinese Food and Drug Administration and the Guangdong Center for Disease Control and Prevention, they have formed the International Cooperation on Alternative Test Methods (ICATM), “working together to promote enhanced international cooperation and coordination on the scientific development, validation and regulatory use of alternative approaches”.²⁸⁷

EURL ECVAM prepares annual reports with updates on the status of alternative methods and approaches. It “provides updates on the progress made in the development, validation and regulatory acceptance and use of alternative methods and approaches and their dissemination”.²⁸⁸

If researchers want a new method to be accepted by regulators, they have to demonstrate that the method is scientifically satisfactory (valid) for the purpose sought. This is done through a validation process.²⁸⁹ Often, the validation process consists of a comparison of the new method with animal tests. This is problematic, because animal tests have not been validated, as argued below:

“While insisting that any non-animal method must undergo validation before it can even be considered for regulatory approval, the pharmaceutical industry and the regulatory authorities have conveniently overlooked the fact that animal experiments have never been validated”.²⁹⁰

“Currently, new in vitro methods are validated against animal models; However, this approach will not help to improve the prediction of the effects of a substance in the human organism in case the animal model is biased”.²⁹¹

“A ... key problem in toxicology may be called the ‘validation dilemma.’ This is posed by the fact that the point of comparison for any novel toxicological tool will be a traditional, poorly assessed methodology. The challenge faced in toxicology is how to objectively assess the value of new tools”.²⁹²

²⁸⁷ European Commission, 2017

²⁸⁸ Zuang, et al., 2018, p. 4

²⁸⁹ Eskes & Whelan, 2016

²⁹⁰ Menache, 2006, p. 5

²⁹¹ Rovida, et al., 2015, p. 179

²⁹² Hartung, 2009a, p. 93

Why it matters

For a long time, the use of animals in research, testing, training and education has been considered a necessary evil. More and more, people question the ethics of this approach. At the same time, the animal research community increasingly recognises the problems with animal research: it is costly, lengthy and not very effective. Also, it may have held back the discovery of treatments and cures for humans because they did not work well in animals.

Long-held beliefs and practices are difficult to change. This is also true for animal research. Besides, there are economic interests at play.²⁹³ But several developments indicate that change is on the way: With an increasing number of people opposed to animal experimentation and in response to public pressure, governments - in particular, in the EU and the US - are encouraging and supporting the development of human-relevant, non-animal research methods. Rapid technological progress enables the development of these new methods.

The main alternatives to the use of animals in the laboratory are new in-silico and in-vitro approaches. Studies with human volunteers and simulators also play an important role. Some of these methods are used in combination for greater effectiveness. Many of the new methods, such as organs-on-chips and organoids, “integrate with in silico approaches and with systems biology, seen by many as having potential to revolutionise medicine and drug discovery”.²⁹⁴ For example, the European Union Reference Laboratory for Alternatives to Animal Testing noted in its 2018 report

that “significant progress has been made in demonstrating how in-vitro and computational methods can be combined to ‘read-across’ toxicological properties between similar chemicals to avoid unnecessary animal testing”.²⁹⁵

So far, most progress in the development of alternatives has been made in the area of toxicology. New tests are being validated and approved by regulators, although this is a slow process and many challenges still lie ahead. But only around 10% of animals are used for regulatory testing. Governments do not require the use of animals for the remaining 90% used in basic and applied research, education and training.

Apart from toxicity testing, the methods and technologies described in this overview can replace animals used in basic and applied research, education and training. As described in the previous sections, there are many applications for the new methods and technologies. They can be used to model and study diseases in general or for individual patients. The new methods can be used to study conditions in individual patients, something that is not possible

with animal models. They have great potential for regenerative and personalised medicine.

The new animal-free methods and technologies are not yet perfect and need further development. Also, some of the current methods that are deemed to be alternatives might still use animal parts or animal data collected in the past. Examples include the tissues or body parts of animals used for food (such as eyes from the slaughterhouse for assessing eye irritation), the use of foetal calf serum for in-vitro cell culture²⁹⁶, or the use of “lesser” animals (such as replacing mammals with zebrafish).²⁹⁷ The databases used for computer-based methods hold data from various sources, and this includes data obtained from previous animal experiments.

There are also other new technologies, such as nanotechnologies and genome editing, that have the potential to replace animal experimentation, but at present they use many animals.²⁹⁸ For this reason, they are not covered in this overview.

²⁹³ e. g., Bottini & Hartung, 2009

²⁹⁴ Pound & Ritskes-Hoitinga, 2018, p. 5

²⁹⁵ Zuang, et al., 2018, p. 1

²⁹⁶ For example, van der Valk & Gstraunthaler, 2017

²⁹⁷ Redmond, 2019

²⁹⁸ Bailey, 2019

Animal researchers argue that the new methods can't replace all areas of current animal research. Given the ethical questions raised and the many shortcomings of animal research, isn't this a compelling argument for speeding up the development of human-relevant research and testing without animals? Governments, the scientific community and industry in EU countries and the US have taken the lead in collaborating to replace animal experimentation. But using animals in research and testing is still the predominant paradigm, the standard way of doing things. Many researchers regard animal testing still as the "gold standard".²⁹⁹

"Why is this so?" one might ask. We know that we are not 70 kg rats³⁰⁰, so why is it so difficult to change this paradigm?

Apart from a reluctance to change, which is common to most humans, a range of other factors are at play. Research funding is competitive, and researchers may not want to take risks by applying for funding for unconventional methods. For example, in Australia only about 15% of National Health and Medical Research Council (NHMRC) grant applications are successful. Unlike in the US and the EU, the Australian government does not provide incentives for new animal-free methods. New methods and technologies also require new skills and new infrastructure. For example, new in-silico methods do not only require proficiency in computer informatics, but scientific expertise in other areas.³⁰¹

Below, two groups of researchers comment on the difficulties of moving away from animal research:

"It is important to recognise that researchers can be reluctant to invest time and money in implementing a new technique, or to replace an animal model that has served as the basis of their research for many years. ... There may be concerns about a lack of historic data comparability, or invalidating past results. Setting up a new model can require additional technical expertise or development of new infrastructure. Referees are familiar with data from the 'gold standard' animal models, and may request additional in vivo data to be generated to support in vitro findings. These factors can delay publication in a highly competitive research environment and result in a lack of motivation to change models".³⁰²

"All believe their research is important and there is an understandable fear to alter methods that have worked well in the past, or to break away from established models linked to previous funding and the published literature. Pressure in the biomedical research field to publish in high-ranking scientific journals can also force researchers to use established models rather than develop better or new models. Current peer review systems do not encourage change to the extent needed".³⁰³

In the near future, industry rather than academic institutions might be more interested and invested in progress towards research and testing without animals. For biotech and pharmaceutical companies, the advantages of new non-animal research methods are compelling – they are cheaper and faster than conventional methods, and more acceptable to customers. Stakeholders in UK drug discovery companies are already calling for more "humanised" preclinical research methods.³⁰⁴ Likewise, a report by Humane Society International stressed the need for change:

"Reflecting the new conceptual approach in safety testing, fundamental health research and drug development need to be based primarily on human disease pathways — not on misplaced efforts to mirror human illnesses in mice and rats. And as safety testing is moving away from simply causing recognisable toxic effects in animals without understanding the toxic pathways involved, so too should research shift its focus from creating artificial 'symptoms' in animals and towards understanding underlying disease pathways in humans".³⁰⁵

We need urgent change. From an animal rights perspective, it was never okay to inflict pain and suffering on animals for the real or perceived benefit of humans. For proponents of animal welfare, the use of animals is justified as long as harm is minimised. With awareness of the many shortcomings of animal research and testing and increasing availability of better ways, the justification for animal research vanishes.

²⁹⁹ Vonk, et al., 2015

³⁰⁰ Hartung, 2009b

³⁰¹ Valerio, 2014

³⁰² Jackson & Thomas, 2017, p. 941

³⁰³ Prescott, Langermans, & Ragan, 2017, p. e2

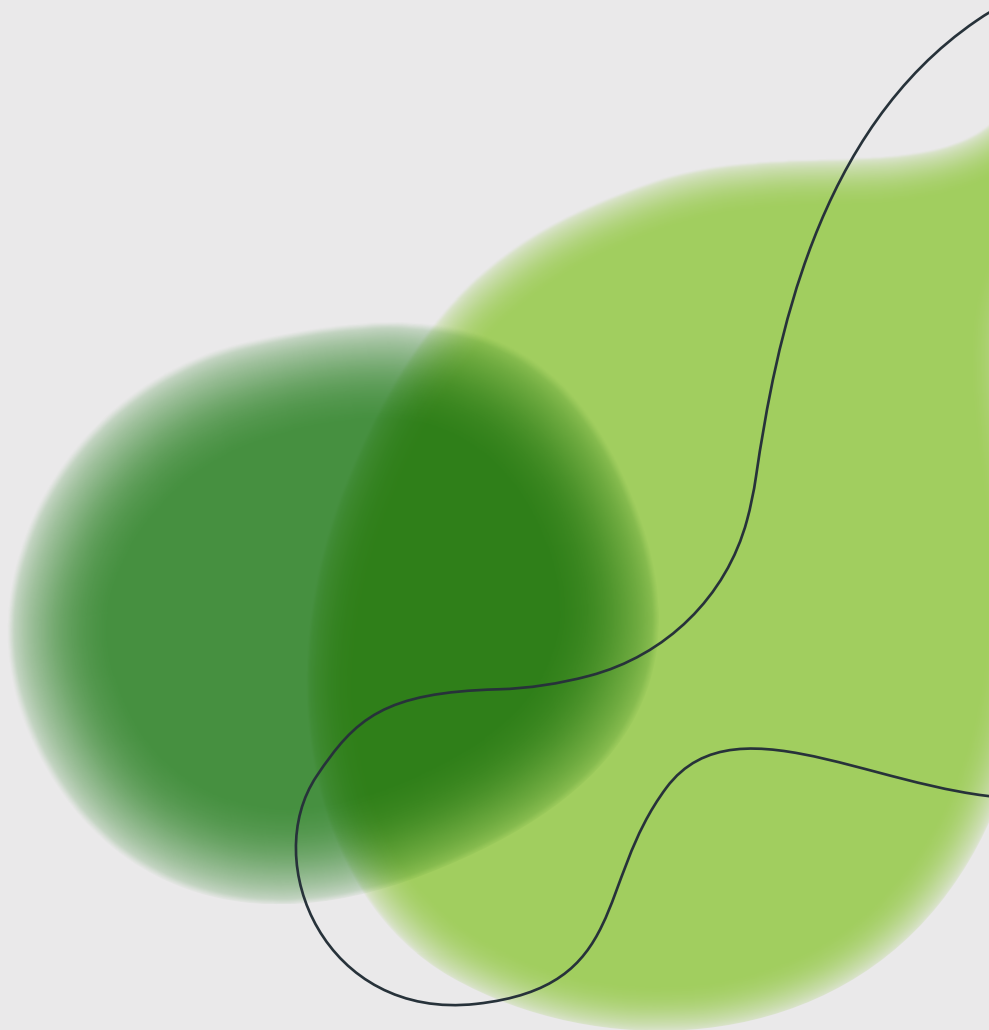
³⁰⁴ Catapult Medicines Discovery and UK BioIndustry Association, 2018

³⁰⁵ Langley, 2012, p. 31

We, the citizens and consumers of health research, need to advocate for better ways to do research and testing. We must persuade our governments to phase out animal research and testing and fund human-relevant and effective research that is based on human biology and not that of animals – research that has real potential to help humans. The new methods and technologies can do more than replacing animals. The future lies in personalised medicine, regenerative medicine, precision medicine, biobanks and personalised drug screening.³⁰⁶

“This new era is marked by a consideration of validity (e.g. reproducibility), by the use of human biomaterials (3D cell cultures, organoids and induced pluripotent stem cells (iPSC)), by the use of ‘high-content’ methods (e.g. Omics), by the combination of computer-based approaches, such as ‘read-across’ and ‘virtual organs’, and by miniaturisation technology (organ/human on a chip)”.³⁰⁷

With greater investment in innovative and promising non-animal methods, firm policy initiatives and robust collaborations of all interested parties, better treatments and cures for human diseases can be developed. This will also end the suffering of millions of animals.



³⁰⁶ e. g., Gorshkov, et al., 2018; Ianelli, 2018

³⁰⁷ Daneshian, 2018, p. 4

Glossary

3Rs

These are the principles of replacement, reduction and refinement. The 3Rs were proposed by William Russell and Rex Burch in the late 1950s and are today embedded in many laws, regulations and codes governing animal use.

- **Replacement** - techniques that replace the use of animals must be sought and used where possible
- **Reduction** - each project must use no more than the minimum number of animals necessary
- **Refinement** - projects should be designed to avoid pain and distress in animals³⁰⁸

Adverse Outcome Pathway (AOP)

"An AOP is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organisation that lead to an adverse health or ecotoxicological effect. ... AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning".³⁰⁹

Animal Ethics Committee (AEC)

AECs oversee the ethical conduct of research using animals. In Australia, AECs include representatives of veterinarians, scientists, animal welfare organisations and the general public. Their primary responsibility is to ensure that all care and use of animals is conducted in compliance with the Australian Code for the Care and Use of Animals for Scientific Purposes.³¹⁰

In other countries these committees may have a different name. For example, in the UK they are called Animal Welfare and Ethical Review Bodies (AWERBs).

Applied research

Applied research is research that seeks to answer a question and/or to solve a practical problem.

Associative network mapping

"Network representations have recently made the leap from social sciences, where they have been applied for decades, to systems biology. Network mappings can provide a picture of the interaction between molecules, represent the relative abundance of those molecules, and provide molecular insights into the organization of signaling pathways, protein-protein interactions, or metabolism, that would not be possible from studying individual proteins or genes. An important finding in many network analyses is that associative networks can elucidate relationships that cannot be seen when comparing single or small sets of genes, proteins, or other components".³¹¹

Bioprinting

"We use the term 3D bioprinting to describe the precise layering of cells, biologic scaffolds, and biologic factors with the goal of recapitulating a biologic tissue. Compared to traditional tissue engineering methods, the technologies utilized by 3D bioprinting systems allow for greater precision in the spatial relationship between the individual elements of the desired tissue".³¹²

Cardiovascular

This term refers to the heart and blood vessels. For example, cardiovascular toxicity refers to damage to the heart and blood vessels by harmful chemicals.

Clinical trials

After pre-clinical research, which involves in-vitro and/or animal studies, promising new drugs or other treatments are tested in clinical trials. The goal is to find out whether the treatment is both safe and effective. Clinical trials involve human volunteers. There are four (sometimes five) phases:

Phase 0 – Usually trials go from pre-clinical research to Phase I, but sometimes a Phase 0 will be run. A very small number of healthy people are given a very small dose of a drug (a microdose). If the drug acts not as expected, additional pre-clinical research will be undertaken.

Phase I – Safety study. Tests a new drug for the first time in healthy humans (unless there was a Phase 0). Includes a small number of volunteers (usually 20-80 people). Shows what side effects the new drug may have. About 70% of medications move on to Phase II.

Phase II – Safety study. Identifies side effects. Tests how well a drug works on a specific disease, such as cancer. Includes a small number of patients (around 100-300 people). About 33% of medications move on to Phase III.

Phase III – Measures effectiveness and monitors side effects. Compares new and standard treatments. Often requires random assignment of the new and standard drugs. Can include hundreds or thousands of patients. Roughly 25% to 30% of medications move on to Phase IV.

If the new drugs passes Phase III, regulatory review and approval follow. The new drug then enters the market.

Phase IV – Monitors long-term side effects after the drug is on the market. Testing for interactions with other drugs. Testing on certain populations, such as pregnant women.

³⁰⁸ National Health and Medical Research Council, 2013

³⁰⁹ OECD, 2018a

³¹⁰ National Health and Medical Research Council, 2013

³¹¹ Marshall, et al., 2018

³¹² Bishop, et al., 2017, p. 186

ECHA

European Chemicals Agency

EU

European Union

EURL ECVAM

European Union Reference Laboratory for alternatives to animal testing

Fundamental research

Also referred to as basic research. This type of research is curiosity driven and not designed to answer specific questions or solve practical problems. Fundamental/basic research is exploratory and aims to increase and advance scientific knowledge.

Haptic feedback

The use of touch to communicate with users. For example, the vibration in a mobile phone.

IATA

Integrated Approach for Testing and Assessment

Immortalised cells

“Immortalized cell lines are either tumorous cells that do not stop dividing or cells that have been artificially manipulated to proliferate indefinitely and can, thus, be cultured over several generations”.³¹³

Incidence

In disease epidemiology, incidence means the number of people newly diagnosed with a condition or disease. In contrast, prevalence is the actual number of people with the disease, both newly diagnosed and diagnosed in the past.

Induced pluripotent stem cells (iPS)

Stem cells which resemble pluripotent embryonic stem cells. They are derived from mature, fully differentiated cells of the body that have been reprogrammed through genetic manipulation and other techniques to restore developmental potential.³¹⁴

In-vitro, in-vivo, in-silico

In-vitro, in-vivo and in-silico are categories of experimental studies. In-vitro (Latin for “within the glass”) experiments are also called test tube experiments. In-vitro research is traditionally done in test tubes, flasks or Petri dishes. More recent in-vitro studies use, for example, organoids and organs-on-chips.

In-vivo means “in the living”. In-vivo methods use living organisms, such as living animals.

In-silico studies are performed on a computer or via computer simulation.

Machine learning

Computer programs that learn from data.

Microfluidic chip

A set of micro-channels etched or moulded into a material, such as glass, silicon or polymer. The micro-channels are connected together. The size of the channels is in the range of one to tens of micrometers. A micrometer is 1000 times smaller than a millimeter.

NHMRC

National Health and Medical Research Council (Australia)

OECD

Organisation for Economic Co-operation and Development

Omics technologies

The term “omics technologies” refers to areas of study in biology whose names end in “omics”, such as genomics (the study of the genome of an organism). The science of “omics” reflects diverse technologies with a focus on studies of life processes, such as comprehensive studies of genes, proteins and metabolites of an organism.

Organoid

A miniature and simplified version of a (human) organ. Organoids are grown in-vitro in three dimensions. They allow researchers to study disease and treatments in the laboratory.

Organ-on-a-chip

“Organs-on-chips are microfluidic devices for culturing living cells in continuously perfused, micrometersized chambers in order to model physiological functions of tissues and organs. The goal is not to build a whole living organ but rather to synthesize minimal functional units that recapitulate tissue- and organ-level functions”.³¹⁵

Peristalsis

Wave-like muscle contractions that move food through the digestive tract.

Pluripotent stem cells

Stem cells that can develop into all types of cells in the body.

³¹³ Carter & Shieh, 2015, p. 298

³¹⁴ Stem Cells Australia, 2018

³¹⁵ Bhatia & Ingber, 2014, p. 760

Physiologically-based pharmacokinetic (PBPK) models

These are “mathematical representations of the absorption, distribution, metabolism and elimination (ADME) of chemicals in humans or other animal species. They are used for multiple purposes, including the interpretation of in vitro toxicity data by in vitro to in vivo extrapolation (IVIVE) and the simulation of internal concentrations in the organism of interest”.³¹⁶

Quantitative structure-activity relationship (QSAR)

“A QSAR is a mathematical model (often a statistical correlation) relating one or more quantitative parameters derived from chemical structure to a quantitative measure of a property or activity (e.g. a (eco)toxicological endpoint). QSARs are quantitative models yielding a continuous or categorical result”.³¹⁷

Read-across structure activity relationships (RASAR)

Machine-learning software combined with big public data sets that can be used to create sophisticated read-across-based tools to predict toxicity of chemical compounds.

REACH

The EU regulation on registration, evaluation, authorisation and restriction of chemicals (REACH).

“REACH is a regulation of the European Union, adopted to improve the protection of human health and the environment from the risks that can be posed by chemicals, while enhancing the competitiveness of the EU chemicals industry. It also promotes alternative methods for the hazard assessment of substances in order to reduce the number of tests on animals”.³¹⁸

Read-across

“‘Read-across and grouping’, or ‘read-across’, is one of the most commonly used alternative approaches for data gap filling in registrations submitted under the REACH Regulation. Read-across involves the use of relevant information from analogous substance(s) (the ‘source’ information) to predict properties for the ‘target’ substance(s) under consideration”.³¹⁹

Regenerative medicine

Regenerative medicine aims at developing methods to regrow, repair or replace damaged or diseased cells, organs or tissues.

Regulatory testing

Testing that is undertaken to comply with relevant laws, policies and (government) regulations.

Stem cells

Unspecialised or undifferentiated cells with the ability to self-renew, and to differentiate to produce specialised cell types in the body.³²⁰

Structure-activity relationship (SAR)

SARs (structure–activity relationships) are mathematical models used for predicting biological activities of chemicals.

“A SAR is a qualitative relationships that relates a (sub)structure to the presence or absence of a property or activity of interest. The substructure may consist of adjacently bonded atoms, or an arrangement of non-bonded atoms that are collectively associated with the property or activity”.³²¹

Systems biology

“An approach that seeks to study organisms as complete systems—networks of interacting genes, biomolecules, and biochemical reactions. It thus attempts to integrate all relevant structural and functional information, rather than focusing on, say, just one particular gene or protein at a time. This involves amassing and organizing data obtained from genomics, proteomics, and other areas of bioinformatics and managing and analysing the data to identify patterns, formulate hypotheses, and ultimately create computer models that will enable accurate predictions of cellular and organismal responses. Such models could have a radical impact on medicine and biology in the future”.³²²

Toxicology

The branch of science concerned with the study of the adverse effects of chemical substances on living organisms and the diagnosis and treatment of exposures to toxins.

US EPA

US Environmental Protection Agency

US FDA

US Food and Drugs Administration

Validation

According to the OECD, validation is defined as “the process by which the reliability and relevance of a particular approach, method, process or assessment is established for a defined purpose”.³²³

Validity

In research, validity is an indication how sound the research is, how well a scientific test or piece of research actually measures what it intends to measure, how well it reflects the reality it claims to represent.

³¹⁶ European Commission EU Science Hub, 2018a

³¹⁷ European Chemicals Agency, 2008, p. 10

³¹⁸ European Chemicals Agency, no year

³¹⁹ European Chemicals Agency, 2017a, p. 4

³²⁰ Stem Cells Australia, 2018

³²¹ European Chemicals Agency, 2008, p. 10

³²² Hine & Martin, 2016

³²³ OECD, 2005, p. 68

References

- Ahadian, S., Civitarese, R., Bannerman, D., Mohammadi, M. H., Lu, R., Wang, E., et al. (2018). Organ-on-a-chip platforms: A convergence of advanced materials, cells, and microscale technologies. *Advanced Healthcare Materials*, 7(2), 1700506-1700506. Available at <https://onlinelibrary.wiley.com/doi/abs/10.1002/adhm.201700506>
- Airman Magazine. (no year). 141024-F-CP197-115. San Antonio Military Medical Center's simulation center. Retrieved 30 January, 2019, from <https://www.flickr.com/photos/airmanmagazine/15843218330/>
- Akçayır, M., & Akçayır, G. (2017). Advantages and challenges associated with augmented reality for education: A systematic review of the literature. *Educational Research Review*, 20, 1-11. Available at <http://www.sciencedirect.com/science/article/pii/S1747938X16300616>
- Akhtar, A. (2015). The flaws and human harms of animal experimentation. *Cambridge Quarterly of Healthcare Ethics*, 24(04), 407-419. Available at <http://dx.doi.org/10.1017/S0963180115000079>
- Alves, V. M., Borba, J., Capuzzi, S. J., Muratov, E., Andrade, C. H., Rusyn, I., et al. (2019). Oy Vey! A comment on "machine learning of toxicological big data enables read-across structure activity relationships outperforming animal test reproducibility". *Toxicological Sciences*, 167(1), 3-4. Available at <https://dx.doi.org/10.1093/toxsci/kfy286>
- AOP-Wiki team. (no year). Collaborative Adverse Outcome Pathway Wiki (AOP-Wiki). Retrieved 11 January, 2019, from <https://aopwiki.org>
- Archibald, K., Tsaion, K., Kenna, J. G., & Pound, P. (2018). Better science for safer medicines: the human imperative. *Journal of the Royal Society of Medicine*, 111(12), 1-6. Available at <https://journals.sagepub.com/doi/abs/10.1177/0141076818812783>
- Ashammakhi, N., Ahadian, S., Zengjie, F., Suthiwanich, K., Lorestani, F., Orive, G., et al. (2018). Advances and future perspectives in 4D bioprinting. *Biotechnology Journal*, 0(ja), 1800148. Available at <https://onlinelibrary.wiley.com/doi/abs/10.1002/biot.201800148>
- Bailey, J. (2019). Genetic modification of animals: Scientific and ethical issues. In K. Herrmann & K. Jayne (Eds.), *Animal experimentation: Working towards a paradigm change* (Vol. 22, pp. 443-478). Leiden, Boston: Brill Human Animal Studies. Available at <https://brill.com/view/book/edcoll/9789004391192/BP000024.xml>
- Baker, E. J., Beck, N. A., Berg, E. L., Clayton-Jeter, H. D., Chandrasekera, P. C., Curley, J. L., et al. (2018). Advancing nonclinical innovation and safety in pharmaceutical testing. *Drug Discovery Today*, 24(2), 624-628. Available at <http://www.sciencedirect.com/science/article/pii/S1359644618302642>
- Bal-Price, A., Leist, M., Schildknecht, S., Tschudi-Monnet, F., Paini, A., & Terron, A. (2018). Adverse Outcome Pathway on inhibition of the mitochondrial complex I of nigro-striatal neurons leading to parkinsonian motor deficits. *OECD*. Retrieved 4 March, 2019, from https://www.oecd-ilibrary.org/environment/adverse-outcome-pathway-on-inhibition-of-the-mitochondrial-complex-i-of-nigro-striatal-neurons-leading-to-parkinsonian-motor-deficits_b46c3c00-en
- Ball, N., Cronin, M., Shen, J., Blackburn, K., Booth, E., Bouhifd, M., et al. (2016). Toward good read-across practice (GRAP) guidance. *ALTEX*, 33(2). Available at <https://www.altex.org/index.php/altex/article/view/167>
- Bhatia, S. N., & Ingber, D. E. (2014). Microfluidic organs-on-chips. *Nature Biotechnology*, 32(8), 760-772. Available at <http://dx.doi.org/10.1038/nbt.2989>
- BioDigital. (2018). The world's first human visualization platform. Anatomy, disease & treatments – all in interactive 3D. Retrieved 24 November, 2018, from <https://www.biodigital.com/>
- Bishop, E. S., Mostafa, S., Pakvasa, M., Luu, H. H., Lee, M. J., Wolf, J. M., et al. (2017). 3-D bioprinting technologies in tissue engineering and regenerative medicine: Current and future trends. *Genes & Diseases*, 4(4), 185-195. Available at <http://www.sciencedirect.com/science/article/pii/S2352304217300673>
- Bottini, A. A., & Hartung, T. (2009). Food for thought... on the economics of animal testing. *Altex*, 26(1), 3-16. Available at <http://www.altex.ch/Current-issue.50.html?iid=104&aid=1>
- Bredenoord, A. L., Clevers, H., & Knoblich, J. A. (2017). Human tissues in a dish: The research and ethical implications of organoid technology. *Science*, 355(6322). Available at <http://science.sciencemag.org/content/sci/355/6322/eaaf9414.full.pdf>
- Brevini, T. A. L., Pennarossa, G., Manzoni, E. F. M., Gandolfi, C. E., Zenobi, A., & Gandolfi, F. (2016). The quest for an effective and safe personalized cell therapy using epigenetic tools. *Clinical Epigenetics*, 8(1), 119. Available at <https://doi.org/10.1186/s13148-016-0283-5>
- Brownell, L. (2018). Taking the brain apart to put it all together again. Retrieved 10 September, 2018, from <https://wyss.harvard.edu/taking-the-brain-apart-to-put-it-all-together-again>
- Carter, M., & Shieh, J. (2015). Chapter 14 - Cell Culture Techniques. In M. Carter & J. Shieh (Eds.), *Guide to Research Techniques in Neuroscience (Second Edition)* (pp. 295-310). San Diego: Academic Press. Available at <http://www.sciencedirect.com/science/article/pii/B9780128005118000149>
- Casati, S. (2018). Integrated approaches to testing and assessment. *Basic & Clinical Pharmacology & Toxicology*, 123(S5), 51-55. Available at <https://onlinelibrary.wiley.com/doi/abs/10.1111/bcpt.13018>
- Catapult Medicines Discovery and UK BioIndustry Association. (2018). *State of the discovery nation 2018 and the role*

- of the Medicines Discovery Catapult. Available at <https://tinyurl.com/ya2cxdxz>
- Chesnut, M., Yamada, T., Adams, T., Knight, D., Kleinstreuer, N., Kass, G., et al. (2018). Regulatory acceptance of read-across. *ALTEX*, 35(3), 413-419. Available at <https://doi.org/10.14573/altex.1805081>
- Cimons, M., Getlin, J., & Maugh, T. H. (1998, 6 May). Cancer drugs face long road from mice to men. *Los Angeles Times*. Available at <http://articles.latimes.com/1998/may/06/news/mn-46795>
- Clark, A. R., Kumar, H., & Burrowes, K. (2017). Capturing complexity in pulmonary system modelling. *Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine*, 231(5), 355-368. Available at <https://journals.sagepub.com/doi/abs/10.1177/0954411916683221>
- Clippinger, A. J., Allen, D., Behrsing, H., Bérubé, K. A., Bolger, M. B., Casey, W., et al. (2018). Pathway-based predictive approaches for non-animal assessment of acute inhalation toxicity. *Toxicology in Vitro*, 52, 131-145. Available at <https://www.sciencedirect.com/science/article/pii/S0887233318302583>
- Clish, C. B. (2015). Metabolomics: an emerging but powerful tool for precision medicine. *Cold Spring Harbor Molecular Case Studies*, 1(1), a000588. Available at <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4850886/>
- Collins, A. R., Annangi, B., Rubio, L., Marcos, R., Dorn, M., Merker, C., et al. (2017). High throughput toxicity screening and intracellular detection of nanomaterials. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, 9(1), e1413. Available at <https://onlinelibrary.wiley.com/doi/abs/10.1002/wnan.1413>
- Colquhoun, R. B., Colquhoun, D. A., & Thiele, R. H. (2011). In silico modelling of physiologic systems. *Best Practice & Research Clinical Anaesthesiology*, 25(4), 499-510. Available at <http://www.sciencedirect.com/science/article/pii/S1521689611000656>
- Contopoulos-Ioannidis, D. G., Ntzani, E. E., & Ioannidis, J. P. A. (2003). Translation of highly promising basic science research into clinical applications. *The American Journal of Medicine*, 114(6), 477-484. Available at [https://doi.org/10.1016/S0002-9343\(03\)00013-5](https://doi.org/10.1016/S0002-9343(03)00013-5)
- Cronin, M., Madden, J. C., Yang, C., & Worth, A. P. (2019). Unlocking the potential of in silico chemical safety assessment – A report on a cross-sector symposium on current opportunities and future challenges. *Computational Toxicology*, 10, 38-43. Available at <http://www.sciencedirect.com/science/article/pii/S2468111318301397>
- Cronin, M., & Yoon, M. (2018). Computational methods to predict toxicity. In M. Balls, R. Combes & A. Worth (Eds.), *The history of alternative test methods in toxicology*. New York: Academic Press. Available at <https://www.elsevier.com/books/the-history-of-alternative-test-methods-in-toxicology/balls/978-0-12-813697-3>
- Cummings, J. L., Morstorf, T., & Zhong, K. (2014). Alzheimer's disease drug-development pipeline: few candidates, frequent failures. *Alzheimer's Research & Therapy*, 6(4), 37-37. Available at <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4095696/>
- Dal Negro, G., Eskes, C., Belz, S., Bertein, C., Chlebus, M., Corvaro, M., et al. (2018). One science-driven approach for the regulatory implementation of alternative methods: A multi-sector perspective. *Regulatory Toxicology and Pharmacology*, 99, 33-49. Available at <http://www.sciencedirect.com/science/article/pii/S0273230018302137>
- Daneshian, M. (2018). *Animal testing belongs to the past – this is the future*. Paper presented at the ALTEX Proceedings. SAP conferences on animal testing, 2016-2018, Olten, Switzerland. Available at http://www.altex.ch/resources/Proceedings_STS_full_issue.pdf
- Daneshian, M., Busquet, F., Hartung, T., & Leist, M. (2015). Animal use for science in Europe. *Altex*, 32(4), 261-274. Available at <https://www.altex.org/index.php/altex/article/view/208>
- Dekkers, J. F., Berkers, G., Kruisselbrink, E., Vonk, A., de Jonge, H. R., Janssens, H. M., et al. (2016). Characterizing responses to CFTR-modulating drugs using rectal organoids derived from subjects with cystic fibrosis. *Science Translational Medicine*, 8(344), 344ra384-344ra384. Available at <http://stm.sciencemag.org/content/scitransmed/8/344/344ra84.full.pdf>
- Designmate (I) Pvt. Ltd. (Producer). (2019, 30 January) Froggipedia. retrieved from <https://itunes.apple.com/us/app/froggipedia/id1348306157>
- Dove, A. (2018). In vitro veritas: Biosensors and microarrays come to life. *Science | AAAS* (14 March). Available at <https://www.sciencemag.org/features/2018/03/vitro-veritas-biosensors-and-microarrays-come-life>
- Duan, B., Hockaday, L. A., Kang, K. H., & Butcher, J. T. (2013). 3D Bioprinting of heterogeneous aortic valve conduits with alginate/gelatin hydrogels. *Journal of Biomedical Materials Research Part A*, 101A(5), 1255-1264. Available at <https://onlinelibrary.wiley.com/doi/abs/10.1002/jbm.a.34420>
- Eskes, C., & Whelan, M. (2016). *Validation of alternative methods for toxicity testing*: Cham : Springer International Publishing : Imprint: Springer.
- European Bank for induced pluripotent Stem Cells. (2018). Welcome to the first European Bank for induced pluripotent Stem Cells (EBISC). Retrieved 30 September, 2018, from <https://www.ebisc.org/>
- European Chemicals Agency. (2008). *Guidance on information requirements and chemical safety assessment. Chapter R.6: QSARs and grouping of chemicals*. Available at https://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf
- European Chemicals Agency. (2017a). *Read-Across Assessment Framework (RAAF)*. Helsinki, Finland: European Chemicals Agency. Available at <https://tinyurl.com/y852eep3>
- European Chemicals Agency. (2017b). *The use of alternatives to testing on animals for the REACH Regulation. Third report under Article 117(3) of REACH*. Helsinki, Finland: ECHA.
- Contopoulos-Ioannidis, D. G., Ntzani,

Available at https://echa.europa.eu/documents/10162/13639/alternatives_test_animals_2017_summary_en.pdf/487e2516-0ad0-90a2-a923-96417ffd6b6b

European Chemicals Agency. (no year). Understanding REACH. Retrieved 6 January, 2019, from <https://echa.europa.eu/regulations/reach/understanding-reach>

European Commission. (2013). *Seventh Report from the Commission to the Council and the European Parliament on the Statistics on the number of animals used for experimental and other scientific purposes in the member states of the European Union*. Brussels, Belgium: European Commission. Available at http://ec.europa.eu/environment/chemicals/lab_animals/reports_en.htm

European Commission. (2017). International Cooperation on Alternative Test Methods (ICATM). Retrieved 17 December, 2018, from <https://ec.europa.eu/jrc/en/eurl/ecvam/alternative-methods-toxicity-testing/advisory-bodies/icatm>

European Commission. (2018a). European Partnership for Alternative Approaches to Animal Testing. Retrieved 4 March, 2019, from https://ec.europa.eu/growth/sectors/chemicals/epaa_en

European Commission. (2018b). European Union Reference Laboratory for Alternatives to Animal Testing. Retrieved 4 March, 2019, from <https://eurl-ecvam.jrc.ec.europa.eu/glossary/glossary/alternative-test-methods>

European Commission. (2018c). *Report from the Commission to the European Parliament and the Council on the development, validation and legal acceptance of methods alternative to animal testing in the field of cosmetics (2015-2017)*. Available at <https://ec.europa.eu/transparency/regdoc/rep/1/2018/EN/COM-2018-531-F1-EN-MAIN-PART-1.PDF>

European Commission EU Science Hub. (2016). Toxtree tool. Retrieved 11 January, 2019, from <https://ec.europa.eu/jrc/en/scientific-tool/toxtree-tool>

European Commission EU Science Hub. (2018a). Computational methods.

Retrieved 11 January, 2019, from <https://ec.europa.eu/jrc/en/eurl/ecvam/alternative-methods-toxicity-testing/computational-methods>

European Commission EU Science Hub. (2018b). Toxmatch. Retrieved 24 February, 2019, from <https://ec.europa.eu/jrc/en/scientific-tool/toxmatch>

European Parliament and the Council of the European Union. (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the European Union* (20.10.2010). Available at <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF>

Feister, A. J., DiPietrantonio, A., Yuenger, J., Ireland, K., & Rao, A. (2018). *Nonhuman primate evaluation and analysis. Part 1: Analysis of future demand and supply*. National Institutes of Health. Available at <https://orip.nih.gov/sites/default/files/508%20NHP%20Evaluation%20and%20Analysis%20Final%20Report%20-%20Part%201.pdf>

Ford, K. A. (2016). Refinement, reduction, and replacement of animal toxicity tests by computational methods. *ILAR Journal*, 57(2), 226-233. Available at <http://dx.doi.org/10.1093/ilar/ilw031>

Franco, N. H. (2013). Animal experiments in biomedical research: A historical perspective. *Animals*, 3(1), 238-273. Available at <http://www.mdpi.com/2076-2615/3/1/238/htm>

Friedrich-Alexander Universitaet Erlangen-Nuernberg. (2018). FAU researchers identify Parkinson's disease as a possible autoimmune disease. Aggressive immune cells aggravate Parkinson's disease. Available at <https://www.fau.eu/2018/07/11/news/research/fau-researchers-identify-parkinsons-disease-as-a-possible-autoimmune-disease/>

Gala, S. G., & Crandall, M. L. (2018). Global collaboration to modernize advanced trauma life support training. *Journal of Surgical Education* (in press). Available at <http://www.sciencedirect.com/science/article/pii/S1931720418304483>

Gomez-Nicola, D., & Boche, D.

(2015). Post-mortem analysis of neuroinflammatory changes in human Alzheimer's disease. *Alzheimer's Research & Therapy*, 7(1), 42. Available at <https://doi.org/10.1186/s13195-015-0126-1>

Gordon, N., & Langley, G. (2008). *Replacing primates in medical research. An expert report by: Dr Hadwen Trust, FRAME, St Andrew Animal Fund*. Available at <http://www.dierproeveninformatie.nl/downloads/Replacing%20primates%20in%20medical%20research.pdf>

Gorshkov, K., Chen, C. Z., Marshall, R. E., Mihatov, N., Choi, Y., Nguyen, D.-T., et al. (2018). Advancing precision medicine with personalized drug screening. *Drug Discovery Today*. Available at <http://www.sciencedirect.com/science/article/pii/S1359644617305378>

Grens, K. (2018). 2013's big advances in science. *The Scientist* (24 December). Available at <https://www.the-scientist.com/research-round-up/2013s-big-advances-in-science-38220>

Haddrick, M., Jones, D., Holmes, A., Hughes, D., Roth Roche, A., Holbrook, M., et al. (2018). *Organ-on-a-chip technologies (OOAC): Current status and translatability of data. Summaries and conclusions of a workshop debate in May 2018 between experts from industry, academia and the Medicines and Healthcare products Regulatory Agency (MHRA) on the subject of OOAC technologies: Medicines Discovery Catapult, Medical Research Council (MRC) Centre for Drug Safety Science (CDSS) University of Liverpool, National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs)*. Available at https://nc3rs.org.uk/sites/default/files/documents/Organ-on-a-chip-technologies_report_FINAL.pdf

Hartung, T. (2009a). 1 Fundamentals of an evidence-based toxicology: 1.1 Opening statement. *Human & Experimental Toxicology*, 28(2-3), 93-94. Available at <https://journals.sagepub.com/doi/abs/10.1177/0960327109105756>

Hartung, T. (2009b). Toxicology for the twenty-first century. *Nature*, 460. Available at <http://dx.doi.org/10.1038/460208a>

- Hartung, T. (2017a). Evolution of toxicological science: the need for change. *International Journal of Risk Assessment and Management*, 20(1/2/3), 21-45. Available at <http://www.inderscience.com/info/inarticle.php?artid=82570>
- Hartung, T. (2017b). Utility of the adverse outcome pathway concept in drug development. *Expert Opinion on Drug Metabolism & Toxicology*, 13(1), 1-3. Available at <https://doi.org/10.1080/17425255.2017.1246535>
- Hartung, T. (2018). AI more accurate than animal testing for spotting toxic chemicals. *The Conversation*. Retrieved from <https://theconversation.com/ai-more-accurate-than-animal-testing-for-spotting-toxic-chemicals-99708>
- Heslop, J. A., & Duncan, S. A. (2018). The use of human pluripotent stem cells for modelling liver development and disease. *Hepatology*(in press). Available at <https://aasldpubs.onlinelibrary.wiley.com/doi/abs/10.1002/hep.30288>
- Hine, R., & Martin, E. (2016). *A dictionary of biology* (7 ed.): Oxford University Press. Available at <http://www.oxfordreference.com/view/10.1093/acref/9780198714378.001.0001/acref-9780198714378-e-6500>
- Home Office. (2015). *Annual statistics of scientific procedures on living animals Great Britain 2014*: UK Government. Available at <https://www.gov.uk/government/statistics/statistics-of-scientific-procedures-on-living-animals-great-britain-2014>
- Hsu, A. C., Starkey, M. R., Hanish, I., Parsons, K., Haw, T. J., Howland, L. J., et al. (2015). Targeting PI3K-p110alpha suppresses influenza virus infection in chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine*, 191(9), 1012-1023.
- Huang, C., Clayton, E. A., Matyunina, L. V., McDonald, L. D., Benigno, B. B., Vannberg, F., et al. (2018). Machine learning predicts individual cancer patient responses to therapeutic drugs with high accuracy. *Scientific Reports*, 8(1), 16444. Available at <https://doi.org/10.1038/s41598-018-34753-5>
- Huang, R., Xia, M., Sakamuru, S., Zhao, J., Lynch, C., Zhao, T., et al. (2018). Expanding biological space coverage enhances the prediction of drug adverse effects in human using in vitro activity profiles. *Scientific Reports*, 8(1), 3783. Available at <https://doi.org/10.1038/s41598-018-22046-w>
- Huh, D., Matthews, B. D., Mammoto, A., Montoya-Zavala, M., Hsin, H. Y., & Ingber, D. E. (2010). Reconstituting organ-level lung functions on a chip. *Science*, 328(5986), 1662-1668. Available at <http://www.sciencemag.org/content/328/5986/1662.abstract>
- Humane Research Australia. (2016). 2016 statistics of animal use in research and teaching. Retrieved 18 January, 2019, from http://www.humanersearch.org.au/statistics/statistics_2016
- Hutchinson, L., & Kirk, R. (2011). High drug attrition rates—where are we going wrong? *Nature Reviews Clinical Oncology*, 8, 189-190. Available at <https://doi.org/10.1038/nrclinonc.2011.34>
- Huys, Q. J. M., Maia, T. V., & Frank, M. J. (2016). Computational psychiatry as a bridge from neuroscience to clinical applications. *Nature Neuroscience*, 19, 404. Available at <https://doi.org/10.1038/nn.4238>
- Ianelli, C. J. (2018). Biobanks: let's share specimens. *Drug Discovery Today*, 23(11), 1809-1811. Available at <http://www.sciencedirect.com/science/article/pii/S1359644618303040>
- Innovate Uk. (2015). *A non-animal technologies roadmap for the UK. Advancing predictive biology*: Innovate UK, the National Centre for the Replacement Refinement and Reduction of Animals in Research, the Biotechnology and Biological Sciences Research Council, the Defence, Science and Technology Laboratory, the Engineering and Physical Sciences Research Council, and the Medical Research Council. Available at https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/474558/Roadmap_NonAnimalTech_final_09Nov2015.pdf
- Institut für Neurowissenschaften und Medizin. (2017). SPM Anatomy toolbox. Retrieved 24 November, 2018, from http://www.fz-juelich.de/inm/inm-1/DE/Forschung/docs/SPMAnatomyToolbox/SPMAnatomyToolbox_node.html
- Interagency Coordinating Committee on the Validation of Alternative Methods. (2018). *A strategic roadmap for establishing new approaches to evaluate the safety of chemicals and medical products in the United States*: National Toxicology Program (NTP), National Institutes of Health. Available at <https://tinyurl.com/yaly9j5m>
- Ionov, L. (2018). 4D biofabrication: Materials, methods, and applications. *Advanced Healthcare Materials*, 7(17), 1800412. Available at <https://onlinelibrary.wiley.com/doi/abs/10.1002/adhm.201800412>
- Jackson, S. J., & Thomas, G. J. (2017). Human tissue models in cancer research: looking beyond the mouse. *Disease Models & Mechanisms*, 10(8), 939-942. Available at <http://dmm.biologists.org/content/10/8/939>
- Judson, R. S., Martin, M. T., Reif, D. M., Houck, K. A., Knudsen, T. B., Rotroff, D. M., et al. (2010). Analysis of eight oil spill dispersants using rapid, in vitro tests for endocrine and other biological activity. *Environmental science & technology*, 44(15), 5979-5985. Available at <https://www.ncbi.nlm.nih.gov/pubmed/20602530>
- Knight, A. (2007). The effectiveness of humane teaching methods in veterinary education. *Altex*, 24(2), 91-109. Available at <http://www.altex.ch/all-issues/issue/2-07/the-effectiveness-of-humane-teaching-methods-in-veterinary-education>
- Langley, G. (2012). *Advancing safety science and health research with innovative, non-animal tools*. London, UK: Humane Society International/Europe. Available at http://www.hsi.org/issues/advancing_science/research/horizon2020report.pdf
- Lappin, G., Noveck, R., & Burt, T. (2013). Microdosing and drug development: past, present and future. *Expert Opinion on Drug Metabolism & Toxicology*, 9(7), 817-834. Available at <https://doi.org/10.1517/17425255.2013.786042>

- Lavelle, G. M., White, M. M., Browne, N., McElvaney, N. G., & Reeves, E. P. (2016). Animal models of cystic fibrosis pathology: Phenotypic parallels and divergences. *BioMed Research International*, 2016, 5258727. Available at <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4908263/>
- Lee, M., Hwang, J.-H., & Lim, K.-M. (2017). Alternatives to in vivo Draize rabbit eye and skin irritation tests with a focus on 3D reconstructed human cornea-like epithelium and epidermis models. *Toxicological Research*, 33(3), 191-203. Available at <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5523559/>
- Leonard, A. V., Thornton, E., & Vink, R. (2014). NK1 receptor blockade is ineffective in improving outcome following a balloon compression model of spinal cord injury. *PLoS ONE*, 9(5), e98364. Available at <http://dx.doi.org/10.1371/journal.pone.0098364>
- Lewis, D. A. (2002). The human brain revisited: Opportunities and challenges in postmortem studies of psychiatric disorders. *Neuropsychopharmacology*, 26, 143. Available at [http://dx.doi.org/10.1016/S0893-133X\(01\)00393-1](http://dx.doi.org/10.1016/S0893-133X(01)00393-1)
- Li, Y.-C., Zhang, Y. S., Akpek, A., Shin, S. R., & Khademhosseini, A. (2017). 4D bioprinting: the next-generation technology for biofabrication enabled by stimuli-responsive materials. *Biofabrication*, 9(1), 012001. Available at <http://stacks.iop.org/1758-5090/9/i=1/a=012001>
- Luechtefeld, T., Marsh, D., Rowlands, C., & Hartung, T. (2018). Machine learning of toxicological big data enables read-across structure activity relationships (RASAR) outperforming animal test reproducibility. *Toxicological Sciences*, 165(1), 198-212. Available at <http://dx.doi.org/10.1093/toxsci/kfy152>
- Luechtefeld, T., Rowlands, C., & Hartung, T. (2018). Big-data and machine learning to revamp computational toxicology and its use in risk assessment. *Toxicology Research*, 7(5), 732-744. Available at <http://dx.doi.org/10.1039/C8TX00051D>
- Maertens, A., & Hartung, T. (2018). Green toxicology—know early about and avoid toxic product liabilities. *Toxicological Sciences*, 161(2), 285-289. Available at <http://dx.doi.org/10.1093/toxsci/kfx243>
- Maertens, A., Hubesch, B., & Rovida, C. (2016). Two good read-across practice workshops. Making it work for you! *ALTEX*, 33(3), 324-326. Available at <https://www.altex.org/index.php/altex/article/view/159>
- Mandt, D., Gruber, P., Markovic, M., Tromayer, M., Rothbauer, M., Krayz, S. R. A., et al. (2018). Fabrication of placental barrier structures within a microfluidic device utilizing two-photon polymerization. *International Journal of Bioprinting*, 4(2). Available at <http://ijb.whioce.com/index.php/int-j-bioprinting/article/view/144>
- Maoz, B. M., Herland, A., FitzGerald, E. A., Grevesse, T., Vidoudez, C., Pacheco, A. R., et al. (2018). A linked organ-on-chip model of the human neurovascular unit reveals the metabolic coupling of endothelial and neuronal cells. *Nature Biotechnology*. Available at <http://dx.doi.org/10.1038/nbt.4226>
- Marshall, L. J., Austin, C. P., Casey, W., Fitzpatrick, S. C., & Willett, C. (2018). Recommendations toward a human pathway-based approach to disease research. *Drug Discovery Today*(online 2 June). Available at <http://www.sciencedirect.com/science/article/pii/S1359644617304737>
- Marshall, L. J., & Willett, C. (2018). Parkinson's disease research: adopting a more human perspective to accelerate advances. *Drug Discovery Today*, 23(12), 1950-1961. Available at <http://www.sciencedirect.com/science/article/pii/S1359644618301442>
- Massa, S., Sakr, M. A., Seo, J., Bandaru, P., Arneri, A., Bersini, S., et al. (2017). Bioprinted 3D vascularized tissue model for drug toxicity analysis. *Biomicrofluidics*, 11(4), 044109. Available at <http://aip.scitation.org/doi/abs/10.1063/1.4994708>
- Mastrangeli, M., Millet, S., Braeken, D., Eberle, W., Luis Fernandez, Graef, M., et al. (2018). *Organ-on-chip in development: Towards a European roadmap for organ-on-chip*. Available at <https://h2020-orchid.eu/wp-content/uploads/2018/12/Towards-a-European-roadmap-for-Organ-on-Chip.pdf>
- McBride, M. T. (2017). Future platforms for toxicity testing. *International Journal of Risk Assessment and Management*, 20(1/2/3), 59-87. Available at <http://www.inderscience.com/info/inarticle.php?artid=82556>
- Mehrban, N., Teoh, G. Z., & Birchall, M. A. (2016). 3D bioprinting for tissue engineering: Stem cells in hydrogels. *International Journal of Bioprinting*, 2(1), 6-19. Available at <http://ijb.whioce.com/index.php/int-j-bioprinting/article/view/02006>
- Meigs, L., Smirnova, L., Rovida, C., Leist, M., & Hartung, T. (2018). Animal testing and its alternatives – the most important omics is economics. *ALTEX*, 35(3). Available at <https://www.altex.org/index.php/altex/article/view/1134>
- Menache, A. (2006). *Non-animal methods of research – who is responsible for holding back progress?* Available at www.humanerresearch.org.au/LiteratureRetrieve.aspx?ID=46580
- Menache, A. (2012). Are animal models relevant in modern psychiatry? . *Psychiatric Times*, 29(3). Available at <http://www.psychiatrictimes.com/psychopharmacology/are-animal-models-relevant-modern-psychiatry>
- Merkes, M., & Buttrorse, R. (2019). Increasing the transparency of animal experimentation: An Australian perspective. In K. Herrmann & K. Jayne (Eds.), *Animal experimentation: Working towards a paradigm change* (Vol. 22, pp. 224-243). Leiden, Boston: Brill Human Animal Studies. Available at <https://brill.com/view/book/edcoll/9789004391192/BP000012.xml>
- Murphy, S. V., & Atala, A. (2014). 3D bioprinting of tissues and organs. *Nature Biotechnology*, 32, 773. Available at <http://dx.doi.org/10.1038/nbt.2958>
- Musah, S., Dimitrakakis, N., Camacho, D. M., Church, G. M., & Ingber, D. E. (2018). Directed differentiation of human induced pluripotent stem cells into mature kidney podocytes and establishment of a Glomerulus Chip. *Nature Protocols*, 13(7), 1662-1685. Available at <https://doi.org/10.1038/s41596-018-0007-8>
- Myatt, G. J., Ahlberg, E., Akahori, Y., Allen, D., Amberg, A., Anger, L. T., et al. (2018). In silico toxicology protocols. *Regulatory Toxicology and*

Pharmacology, 96, 1-17. Available at <http://www.sciencedirect.com/science/article/pii/S0273230018301144>

National Academies of Sciences, E., and Medicine,. (2017). *Using 21st century science to improve risk-related evaluations*. Washington, DC: The National Academies Press. Available at <https://www.nap.edu/catalog/24635/using-21st-century-science-to-improve-risk-related-evaluations>

National Center for Advancing Translational Sciences. (2018a). 3-D image of bioprinted skin tissue. Retrieved 30 January, 2019, from <https://www.flickr.com/photos/64860478@N05/28746740398/>

National Center for Advancing Translational Sciences. (2018b). About new therapeutic uses. Retrieved 15 June, 2018, from <https://ncats.nih.gov/ntu/about>

National Center for Advancing Translational Sciences. (2018c). Heart-on-a-chip. Retrieved 30 January, 2019, from <https://www.flickr.com/photos/64860478@N05/27460050987/>

National Center for Advancing Translational Sciences. (2018d). Multiple connected chips. Credit: NCATS. Retrieved 30 January, 2019, from <https://www.flickr.com/photos/64860478@N05/41427884275/in/photostream/>

National Center for Advancing Translational Sciences. (2018e). Neural stem cells in 3-D culture models. Retrieved 30 January, 2019, from <https://www.flickr.com/photos/64860478@N05/41556744195/in/photostream/>

National Center for Advancing Translational Sciences. (2018f). Spinal cord nerve and blood vessel cells on a tissue chip. Retrieved 30 January, 2019, from <https://www.flickr.com/photos/64860478@N05/41772508334/in/photostream/>

National Center for Advancing Translational Sciences. (no year-a). 3-D printed eye tissue. Retrieved 30 January, 2019, from <https://www.flickr.com/photos/64860478@N05/34100509955/>

National Center for Advancing Translational Sciences. (no year-b). Blood-brain barrier on a chip. Credit: Vanderbilt University. Retrieved 30 January, 2019, from <https://www.flickr.com/photos/64860478@N05/27475445617/>

National Center for Advancing Translational Sciences. (no year-c). Kidney-on-a-chip. Retrieved 30 January, 2019, from <https://www.flickr.com/photos/64860478@N05/40541709610/>

National Center for Advancing Translational Sciences. (no year-d). Lung-on-a-chip. Credit: Wyss Institute for Biologically Inspired Engineering, Harvard University. Retrieved 30 January, 2019, from <https://www.flickr.com/photos/64860478@N05/27479210827/>

National Center for Advancing Translational Sciences. (no year-e). Organovo BioPrinter. Retrieved 30 January, 2019, from <https://www.flickr.com/photos/64860478@N05/42429024092/in/photostream/>

National Health and Medical Research Council. (2013). *Australian code for the care and use of animals for scientific purposes*. 8th Edition 2013. Canberra: NHMRC, Australian Government. Available at <https://tinyurl.com/y7he6lxm>

National Institute for Public Health and the Environment. (no year). EuroMix. A tiered strategy for risk assessment of mixtures of multiple chemicals. Retrieved 1 March, 2019, from <https://www.euromixproject.eu>

National Institutes of Health. (2016). Stem cell information. Retrieved 23 September, 2018, from <https://stemcells.nih.gov/info/basics/7.htm>

Navarro, A. M., Susanto, E., Falk, A., & Wilhelm, M. (2018). Modeling cancer using patient-derived induced pluripotent stem cells to understand development of childhood malignancies. *Cell Death Discovery*, 4(1), 7. Available at <https://doi.org/10.1038/s41420-017-0009-2>

Netherlands National Committee for the protection of animals used for

scientific purposes. (2016). *Transition to non-animal research. On opportunities for the phasing out of animal procedures and the stimulation of innovation without laboratory animals*. The Hague, The Netherlands: NCad. Available at <https://tinyurl.com/y7l5dca3>

Nielsen, K. N., Peics, J., Ma, T., Karavaeva, I., Dall, M., Chubanova, S., et al. (2018). NAMPT-mediated NAD⁺ biosynthesis is indispensable for adipose tissue plasticity and development of obesity. *Molecular Metabolism*, 11, 178-188. Available at <http://www.sciencedirect.com/science/article/pii/S2212877817310773>

NIH Image Gallery. (2018). Inner ear organoid. Retrieved 12 April, 2019, from <https://www.flickr.com/photos/nihgov/41211354025/in/photolist-25MGXLD-oy5jTd>

NIH Image Gallery. (2019). Brain organoid. Retrieved 12 April, 2019, from <https://www.flickr.com/photos/nihgov/32829360978/>

OECD. (2005). *Guidance document on the validation and international acceptance of new or updated test methods for hazard assessment*. Series on Testing and Assessment, No. 34. Paris: OECD. Available at <https://tinyurl.com/ycefpao3>

OECD. (2016). Effectopedia. The online encyclopedia of adverse outcome pathways. Retrieved 11 January, 2019, from <https://www.effectopedia.org>

OECD. (2018a). Adverse outcome pathways, molecular screening and toxicogenomics. Retrieved 11 January, 2019, from <http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm>

OECD. (2018b). Integrated Approaches to Testing and Assessment (IATA). Retrieved 1 March, 2019, from <http://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm>

OECD. (2018c). The OECD QSAR Toolbox. Retrieved 7 January, 2019, from <http://www.oecd.org/chemicalsafety/risk-assessment/oecd->

qsar-toolbox.htm

OECD. (2019). Adverse Outcome Pathway Knowledge Base (AOP-KB). Retrieved 25 February, 2019, from <https://aopkb.oecd.org/background.html>

Paini, A., Leonard, J. A., Joossens, E., Bessems, J. G. M., Desalegn, A., Dorne, J. L., et al. (2019). Next generation physiologically based kinetic (NG-PBK) models in support of regulatory decision making. *Computational Toxicology*, 9(February), 61-72. Available at <http://www.sciencedirect.com/science/article/pii/S246811131830077X>

Paraizo Leite, R. E., & Tenenholz Grinberg, L. (2015). Closing the gap between brain banks and proteomics to advance the study of neurodegenerative diseases. *Proteomics. Clinical Applications*, 9(9-10), 832-837.

Park, J. Y., Jang, J., & Kang, H.-W. (2018). 3D Bioprinting and its application to organ-on-a-chip. *Microelectronic Engineering*, 200, 1-11. Available at <http://www.sciencedirect.com/science/article/pii/S0167931718304027>

Park, K. M., Shin, Y. M., Kim, K., & Shin, H. (2018). Tissue engineering and regenerative medicine 2017: A year in review. *Tissue Engineering Part B: Reviews*, 24(5), 327-344. Available at <https://www.liebertpub.com/doi/abs/10.1089/ten.teb.2018.0027>

Park, T. H., & Shuler, M. L. (2003). Integration of cell culture and microfabrication technology. *Biotechnology Progress*, 19(2), 243-253. Available at <https://onlinelibrary.wiley.com/doi/abs/10.1021/bp020143k>

Parton, A., McGilligan, V., O'Kane, M., Baldrick, F. R., & Watterson, S. (2016). Computational modelling of atherosclerosis. *Briefings in Bioinformatics*, 17(4), 562-575. Available at <http://dx.doi.org/10.1093/bib/bbv081>

Passini, E., Britton, O. J., Lu, H. R., Rohrbacher, J., Hermans, A. N., Gallacher, D. J., et al. (2017). Human in silico drug trials demonstrate higher accuracy than animal models in predicting clinical pro-arrhythmic cardiotoxicity. *Frontiers in Physiology*, 8(668). Available at <http://journal.frontiersin.org/article/10.3389/>

fphys.2017.00668

Passini, E., Rodriguez, B., & Benito, P. (2018). Why computer simulations should replace animal testing for heart drugs. Retrieved 14 January, 2019, from <https://theconversation.com/why-computer-simulations-should-replace-animal-testing-for-heart-drugs-93409>

Patlewicz, G., Ball, N., Becker, R. A., Booth, E. D., Cronin, M. T., Kroese, D., et al. (2014). Food for thought ... Read-across approaches - misconceptions, promises and challenges ahead. *Altex*, 31(4), 387-396. Available at <https://www.altex.org/index.php/altex/article/view/275/275>

Pelargos, P. E., Nagasawa, D. T., Lagman, C., Tenn, S., Demos, J. V., Lee, S. J., et al. (2017). Utilizing virtual and augmented reality for educational and clinical enhancements in neurosurgery. *Journal of Clinical Neuroscience*, 35, 1-4. Available at <http://www.sciencedirect.com/science/article/pii/S0967586816303162>

PETA. (2019). PETA International Science Consortium Ltd. Retrieved 15 January, 2019, from <https://www.piscltd.org.uk>

Pfandler, M., Lazarovici, M., Stefan, P., Wucherer, P., & Weigl, M. (2017). Virtual reality-based simulators for spine surgery: a systematic review. *The Spine Journal*, 17(9), 1352-1363. Available at <https://doi.org/10.1016/j.spinee.2017.05.016>

Pluijmert, M., Lumens, J., Potse, M., Delhaas, T., Auricchio, A., & Prinzen, F. W. (2015). Computer modelling for better diagnosis and therapy of patients by cardiac resynchronisation therapy. *Arrhythmia & Electrophysiology Review*, 4(1), 62-67. Available at <https://www.ncbi.nlm.nih.gov/pubmed/26835103>

Pound, P., & Ritskes-Hoitinga, M. (2018). Is it possible to overcome issues of external validity in preclinical animal research? Why most animal models are bound to fail. *Journal of Translational Medicine*, 16(1), 304. Available at <https://doi.org/10.1186/s12967-018-1678-1>

Prescott, M. J., Langermans, J. A., & Ragan, I. (2017). Applying the 3Rs to non-human primate research: Barriers and solutions. *Drug Discovery Today: Disease Models*(21 November). Available

at <http://www.sciencedirect.com/science/article/pii/S1740675717300385>

Probst, C., Schneider, S., & Loskill, P. (2018). High-throughput organ-on-a-chip systems: Current status and remaining challenges. *Current Opinion in Biomedical Engineering*, 6(June), 33-41. Available at <http://www.sciencedirect.com/science/article/pii/S2468451117300909>

Quick, J. A. (2018). Simulation training in trauma. *Missouri medicine*, 115(5), 447-450. Available at <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6205286/>

Raies, A. B., & Bajic, V. B. (2016). In silico toxicology: computational methods for the prediction of chemical toxicity. *Wiley interdisciplinary reviews. Computational molecular science*, 6(2), 147-172. Available at <https://www.ncbi.nlm.nih.gov/pubmed/27066112>

Ram, R. (2019). Extrapolation of animal research data to humans: An analysis of the evidence. In K. Herrmann & K. Jayne (Eds.), *Animal experimentation: Working towards a paradigm change* (Vol. 22, pp. 341-375). Leiden, Boston: Brill Human Animal Studies. Available at <https://brill.com/view/book/edcoll/9789004391192/BP000020.xml>

Ranganatha, N., & J, K. I. (2012). A review on alternatives to animal testing methods in drug development. *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(Suppl 5), 28-32. Available at <https://pdfs.semanticscholar.org/8440/666fb4012bcd6b6f23b86a1c9a28db214e6f.pdf>

Redmond, C. (2019). When is an alternative not an alternative? Supporting progress for absolute replacement of animals in science. In K. Herrmann & K. Jayne (Eds.), *Animal experimentation: Working towards a paradigm change* (Vol. 22, pp. 654-672). Leiden, Boston: Brill. Available at <https://brill.com/view/title/35072>

RepoTrial. (2018). REPO-TRIAL: Setting standards for in silico drug repurposing. Retrieved 28 February, 2019, from <https://repo-trial.eu>

Rothbauer, M., Rosser, J. M., Zirath, H., & Ertl, P. (2019). Tomorrow today: organ-on-a-chip advances towards clinically relevant pharmaceutical and medical in vitro models. *Current Opinion in*

Biotechnology, 55, 81-86. Available at <http://www.sciencedirect.com/science/article/pii/S0958166918300570>

Rovida, C., Asakura, S., Daneshian, M., Hofman-Huether, H., Leist, M., Meunier, L., et al. (2015). Toxicity testing in the 21st century beyond environmental chemicals. *Altex*, 32(3), 171-181. Available at <https://www.altex.org/index.php/altex/article/view/222>

Rowland, M. (2012). Microdosing: A critical assessment of human data. *Journal of Pharmaceutical Sciences*, 101(11), 4067-4074. Available at <https://onlinelibrary.wiley.com/doi/abs/10.1002/jps.23290>

Rzeznik, M., Triba, M. N., Levy, P., Jungo, S., Botosoa, E., Duchemann, B., et al. (2017). Identification of a discriminative metabolomic fingerprint of potential clinical relevance in saliva of patients with periodontitis using ¹H nuclear magnetic resonance (NMR) spectroscopy. *PLOS ONE*, 12(8), e0182767. Available at <https://doi.org/10.1371/journal.pone.0182767>

Savoji, H., Mohammadi, M. H., Rafatian, N., Toroghi, M. K., Wang, E. Y., Zhao, Y., et al. (2018). Cardiovascular disease models: A game changing paradigm in drug discovery and screening. *Biomaterials* (in press). Available at <http://www.sciencedirect.com/science/article/pii/S0142961218306811>

Seok, J., Warren, H. S., Cuenca, A. G., Mindrinos, M. N., Baker, H. V., Xu, W., et al. (2013). Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proceedings of the National Academy of Sciences*, 110(9), 3507-3512. Available at <http://www.pnas.org/content/early/2013/02/07/1222878110.abstract>

Simulab. (2017). TraumaMan never grows old. Retrieved 25 November, 2018, from <https://www.simulab.com/traumaman/about>

Singer, P. (1989). Animal liberation: A summary statement. *Island Magazine*, 38, 36-38.

Sinha, G. (2017, 23 August). This scientist is building miniature guts, livers, and lungs that could save your life one day. *Science | AAAS*. Available at <https://www.sciencemag.org/news/2017/08/scientist-building-miniature-guts-livers-and-lungs-could>

save-your-life-one-day

Slesnick, T. C. (2017). Role of computational modelling in planning and executing interventional procedures for congenital heart disease. *Canadian Journal of Cardiology*, 33(9), 1159-1170. Available at <http://www.sciencedirect.com/science/article/pii/S0828282X1730274X>

Slikker, W., de Souza Lima, T. A., Archella, D., de Silva, J. B., Barton-Maclaren, T., Bo, L., et al. (2018). Emerging technologies for food and drug safety. *Regulatory Toxicology and Pharmacology*, 98, 115-128. Available at <http://www.sciencedirect.com/science/article/pii/S0273230018301971>

Sommer, A., Maxreiter, F., Krach, F., Fadler, T., Grosch, J., Maroni, M., et al. (2018). Th17 lymphocytes induce neuronal cell death in a human iPSC-based model of Parkinson's Disease. *Cell Stem Cell*, 23(1), 123-131.e126. Available at <http://dx.doi.org/10.1016/j.stem.2018.06.015>

Sriram, G., Alberti, M., Dancik, Y., Wu, B., Wu, R., Feng, Z., et al. (2018). Full-thickness human skin-on-chip with enhanced epidermal morphogenesis and barrier function. *Materials Today*, 21(4), 326-340. Available at <http://www.sciencedirect.com/science/article/pii/S1369702117304844>

Stem Cells Australia. (2018). About stem cells. Retrieved 7 September, 2018, from <http://www.stemcellsaustralia.edu.au/About-Stem-Cells.aspx>

Strauss, M. (2018). Americans are divided over the use of animals in scientific research. Retrieved 1 November, 2018, from <http://www.pewresearch.org/fact-tank/2018/08/16/americans-are-divided-over-the-use-of-animals-in-scientific-research/>

Struillou, X., Boutigny, H., Soueidan, A., & Layrolle, P. (2010). Experimental animal models in periodontology: A review. *The Open Dentistry Journal*, 4, 37-47. Available at <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2885595/>

Svendsen, P., El-Galaly, T. C., Dybkær, K., Bøgsted, M., Laursen, M. B., Schmitz, A., et al. (2016). The application of human phase 0 microdosing trials: A systematic review and perspectives. *Leukemia & Lymphoma*, 57(6), 1281-1290. Available at <https://doi.org/10.31>

09/10428194.2015.1101097

Taylor, K. (2019). Recent developments in alternatives to animal testing. In K. Herrmann & K. Jayne (Eds.), *Animal experimentation: Working towards a paradigm change* (Vol. 26, pp. 585-609). Leiden, Boston: Brill. Available at <https://brill.com/view/title/35072>

Taylor, K., Gordon, N., Langley, G., & Higgins, W. (2008). Estimates for worldwide laboratory animal use in 2005. *Alternatives to laboratory animals: ATLA*, 36(3), 327-342. Available at https://crueltyfreeinternational.org/sites/default/files/Tayloretal_worlddestimates_2008_%20ATLA.pdf

The Hastings Center. (no date). Fact sheet. Animals used in research in the U.S. Retrieved 4 March, 2019, from <http://animalresearch.thehastingscenter.org/facts-sheets/animals-used-in-research-in-the-united-states/>

Thomas, D. W., Burns, J., Audette, J., Carroll, A., Dow-Hygelund, C., & Hay, M. (2016). *Clinical development success rates 2006-2015*: Amplion, Biomedtracker and Biotechnology Innovation Organization. Available at <https://www.bio.org/sites/default/files/Clinical%20Development%20Success%20Rates%202006-2015%20-%20BIO,%20Biomedtracker,%20Amplion%202016.pdf>

Thomson, J. E., Poudrier, G., Stranix, J. T., Motosko, C. C., & Hazen, A. (2018). Current status of simulation training in plastic surgery residency programs: A review. *Archives of Plastic Surgery*, 45(5), 395-402. Available at <https://doi.org/10.5999/aps.2017.01585>

Timoshanko, A. C., Marston, H., & Lidbury, B. A. (2017). Australian regulation of animal use in science and education: A critical appraisal *ILAR Journal*, 57(3), 324-332. Available at <https://academic.oup.com/ilarjournal/article-abstract/57/3/324/3796585/Australian-Regulation-of-Animal-Use-in-Science-and>

Triunfol, M., Rehen, S., Simian, M., & Seidle, T. (2018). Human-specific approaches to brain research for the 21st century: a South American perspective. *Drug Discovery Today*, 23(12). Available at <https://www.sciencedirect.com/science/article/pii/>

[S1359644617304749](#)

U.S. Environmental Protection Agency. (2017). Tox21. Toxicology in the 21st Century. Retrieved 5 January, 2018, from <https://tinyurl.com/ybt92dat>

U.S. Food and Drug Administration. (2017). *FDA's predictive toxicology roadmap*: FDA. Available at <https://tinyurl.com/y8jcvtoc>

Underwriters Laboratories. (no year). Are you looking for a rapid and accurate approach to meet your REACH compliance needs? Retrieved 4 March, 2019, from <https://www.ulreachacross.com/>

United States Environmental Protection Agency. (2018a). New GenRA module in EPA's CompTox Dashboard will help predict potential chemical toxicity. Retrieved 14 January, 2019, from <https://www.epa.gov/sciencematters/new-genra-module-epas-comptox-dashboard-will-help-predict-potential-chemical-toxicity>

United States Environmental Protection Agency. (2018b). What is Toxicology in the 21st Century (Tox21)? Retrieved 10 January, 2019, from <https://www.epa.gov/chemical-research/what-toxicology-21st-century-tox21>

Valerio, L. G. (2014). In silico methods. In P. Wexler (Ed.), *Encyclopedia of Toxicology (Third Edition)* (pp. 1026-1029). Oxford: Academic Press. Available at <http://www.sciencedirect.com/science/article/pii/B9780123864543010575>

van de Burgwal, L. H. M., van Dorst, P., Viëtor, H., Luttge, R., & Claassen, E. (2018). Hybrid business models for 'Organ-on-a-Chip' technology: The best of both worlds. *PharmaNutrition*, 6(2), 55-63. Available at <http://www.sciencedirect.com/science/article/pii/S2213434417301147>

van der Valk, J., & Gstraunthaler, G. (2017). Fetal Bovine Serum (FBS) - A pain in the dish? *Alternatives to Laboratory Animals*, 45(6), 329-332. Available at <http://www.atla.org.uk/fetal-bovine-serum-fbs-a-pain-in-the-dish/>

van der Worp, H. B., Howells, D. W., Sena, E. S., Porritt, M. J., Rewell, S., O'Collins, V., et al. (2010). Can animal

models of disease reliably inform human studies? *PLoS Medicine*, 7(3), e1000245. Available at <http://dx.doi.org/10.1371/journal.pmed.1000245>

Vaughan, N., Dubey, V. N., Wainwright, T. W., & Middleton, R. G. (2016). A review of virtual reality based training simulators for orthopaedic surgery. *Medical Engineering and Physics*, 38(2), 59-71. Available at <https://doi.org/10.1016/j.medengphy.2015.11.021>

Viceconti, M., & Hunter, P. (2016). The Virtual Physiological Human: Ten years after. *Annual Review of Biomedical Engineering*, 18(1), 103-123. Available at <https://www.annualreviews.org/doi/abs/10.1146/annurev-bioeng-110915-114742>

Vijayavenkataraman, S., Yan, W.-C., Lu, W. F., Wang, C.-H., & Fuh, J. Y. H. (2018). 3D bioprinting of tissues and organs for regenerative medicine. *Advanced Drug Delivery Reviews*. Available at <http://www.sciencedirect.com/science/article/pii/S0169409X18301686>

Vonk, R. A. A., van de Laar, C. W. E., Hegger, I., Ezendam, J., Janssen, S. W. J., & Hoebert, J. M. (2015). *Legal barriers for the use of alternatives to animal testing: do current EU regulations and guidelines for regulatory acceptance of medicinal products pose legal barriers? RIVM Letter report 2015-0084*. Netherlands: National Institute for Public Health and the Environment, Ministry of Health, Welfare and Sport. Available at <https://tinyurl.com/y3t9xezo>

Wilkinson, M. (2019). The potential of organ on chip technology for replacing animal testing. In K. Herrmann & K. Jayne (Eds.), *Animal experimentation: Working towards a paradigm change* (Vol. 26, pp. 639-653). Leiden, Boston: Brill. Available at <https://brill.com/view/title/35072>

Worth, A., & Blaauboer, B. (2018). Chapter 5.4. Integrated Approaches to Testing and Assessment. In M. Balls, R. Combes & A. Worth (Eds.), *The history of alternative test methods in toxicology* (pp. 301-306). New York: Academic Press. Available at <https://www.elsevier.com/books/the-history-of-alternative-test-methods-in-toxicology/balls/978-0-12-813697-3>

Xu, F., Celli, J., Rizvi, I., Moon, S., Hasan, T., & Demirci, U. (2011). A three-dimensional in vitro ovarian cancer coculture model using a high-throughput cell patterning platform. *Biotechnology Journal*, 6(2), 204-212. Available at <https://onlinelibrary.wiley.com/doi/abs/10.1002/biot.201000340>

Yaacov, L. (2015). MSR simulator. Retrieved 30 January, 2019, from <https://commons.wikimedia.org/w/index.php?curid=55338800>

Yadav, D., Tanveer, A., Malviya, N., & Yadav, S. (2017). Overview and principles of bioengineering: The drivers of omics technologies. In D. Barh & V. Azevedo (Eds.), *Omics technologies and bio-engineering : Towards improving quality of life*: Elsevier Science & Technology.

Zhang, B., Korolj, A., Lai, B. F. L., & Radisic, M. (2018). Advances in organ-on-a-chip engineering. *Nature Reviews Materials*, 3(8), 257-278. Available at <https://doi.org/10.1038/s41578-018-0034-7>

Zhu, H., Bouhifd, M., Donley, E., Egnash, L., Kleinstreuer, N., Kroese, E. D., et al. (2016). Supporting read-across using biological data. *Altex*, 33(2), 167-182. Available at <https://www.altex.org/index.php/altex/article/view/168>

Zuang, V., Dura, A., Asturiol, B. D., Viegas, B. J. F., Batista, L. S., Belz, S., et al. (2018). *EURL ECVAM status report on the development, validation and regulatory acceptance of alternative methods and approaches* (2018). Luxembourg: European Union. Available at http://publications.jrc.ec.europa.eu/repository/bitstream/JRC113594/eurl_ecvam_status_report_2018_online.pdf

Zuang, V., Viegas Barroso, J. F., Belz, S., Berggren, E., Bernasconi, C., Bopp, S., et al. (2017). *EURL ECVAM status report on the development, validation and regulatory acceptance of alternative methods and approaches* (2017). Luxembourg: European Commission. Available at <http://publications.jrc.ec.europa.eu/repository/handle/JRC108831>

