2025

ANIMAL TESTING LANDSCAPE

ANIMALS IN SCIENCE & ALTERNATIVES

CHERRY BIOTECH

Table of Content

Introduction

For centuries, experimental medicine has played a crucial role in medical progress, notably through animal experimentation. These have led to major discoveries that have saved countless lives.

It has contributed to our understanding of many diseases, and to the development of treatments that would otherwise have been unimaginable.

Illustrating the tension and ethical debate within society, it is interesting to recall that Fanny Martin - the wife of Claude Bernard (one of the fathers of experimental medicine), spent much of her life in animal protection.

As science progresses, so do techniques, and traditional methods of animal experimentation are no longer necessarily best suited to the modern challenges of biomedical research. Today, there are alternatives that are more precise, less costly and more ethical.

Although regulatory changes are underway to promote these new methods, their adoption and the subsequent scientific results, the superiority of these approaches remains too slow in view of the immense potential they offer.

What is our greatest challenge?

In his fundamental work “The Structure of Scientific Revolutions” (1962), Thomas Kuhn describes how science progresses not linearly, but through periods of continuity alternating with radical ruptures, which he calls scientific revolutions.

In 2025, at a time when the rapid progress of AI is more than ever questioning our notions of consciousness, sensitivity, and autonomy it seems essential to decide on the proper use of technologies and to reconsider our current and future relationship with laboratory animals.

As manufacturer of alternatives and actors in scientific research, we present this introductory, non-exhaustive document as an overview of animal experimentation, regulatory trends, and existing alternatives.

We hope it will help bring about these “ruptures” and make scientific research more ethical and predictive in humans.

Happy reading.

Jérémy Cramer
Founder and CEO at Cherry Biotech.

OVERVIEW

The current outlook of animal testing and experiments worldwide and pan European, with factsheets

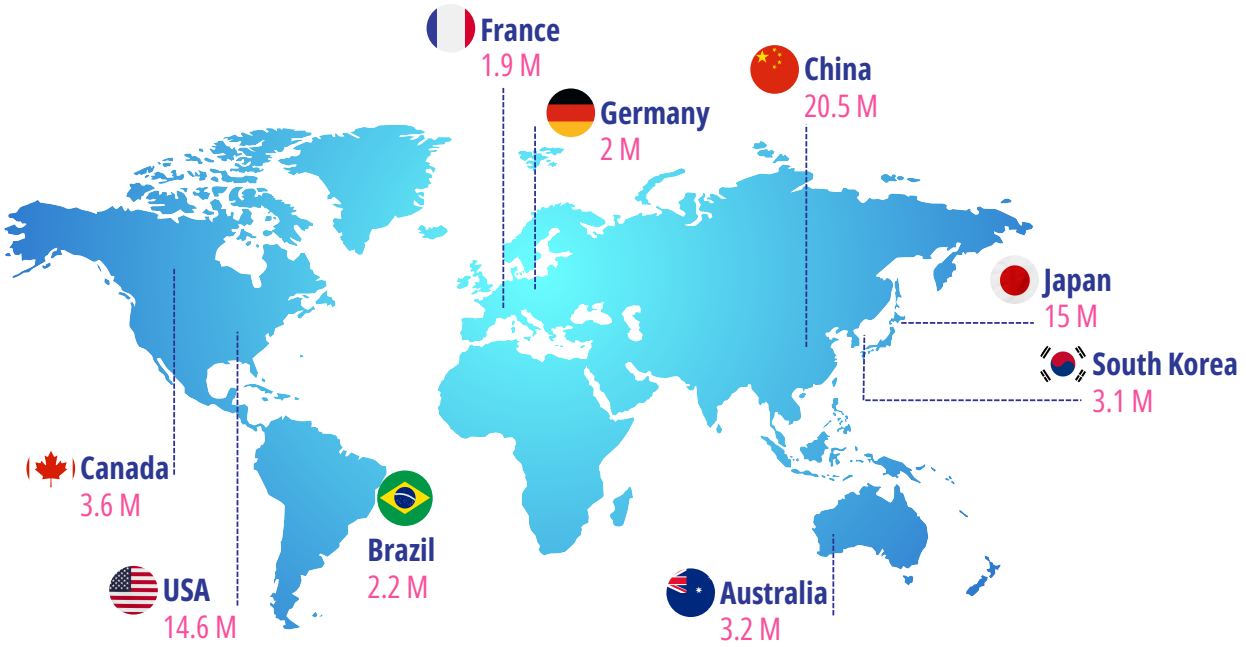
Taylor and Alvarez (2019) [1] conducted the most accurate study to date to estimate the annual global use of animals in research.

Their methodology involved standardizing data from 37 countries that publish national statistics on animal use, along with a prediction model based on the number of scientific publications involving animals.

This approach yielded a comprehensive global estimate of 192.1 million animals used for scientific purposes.

Taylor and Alvarez conducted a similar study in 2005 and estimated the use of approximately 115 million animals (a 67% increase between the two studies).

Top 10 animal-using countries



Top 10 countries : 68.7m
Rest of the world : 11.2m
Total : 79.9m

Source: Taylor, K., & Alvarez, L. R. (2019)[1]

What is the gap between 192 and 79.9 million ?

Taylor and Alvarez study estimates approximately 192 millions animal used for science every year. However, in the same study the sum of animals used by every country "only" reaches 79.9 millions. **What explains this gap ?**

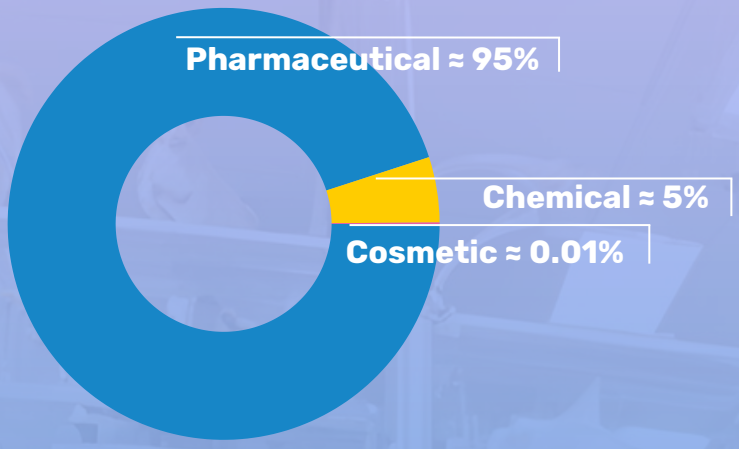
To calculate the total number of animal used for science Taylor and Alvarez include animals that are not directly used for research but for "logistic" purposes :

- Tissue supply
- Maintaining genetically modified (GM) colonies
- Surplus animals bred but not used.

They have estimated that **for every 1 animal used in science, there are 1.4 additional animals used for "logistic" purposes**. So for 79,9 million animals used in experimentation, there are 79.9 x (1+1.4) = 192 millions animal used in total.

This gap is important to keep in mind when we hear the number of animals being used in experiments. Behind this number, there is an even bigger number of animals used for "logistic" purposes.

Which industries perform tests on animals ?



95% of animals in science are used by the **pharmaceutical industry.** This usage will be detailed on the next pages.

Chemical Industry

Around 6 million animals worldwide [2] are estimated to be used by the chemical industry. These animals are used to evaluate the toxicity of chemical products before allowing them on the market.
Among the types of product for which the chemical industry uses animals :

- Paints
- Dyes
- Plastics
- Pesticides
- Household cleaners
- Food additives

Although regulations like REACH [3] in Europe and TSCA [4] in the US promote the use of alternatives, there are no global bans on testing chemical products on animals.

Cosmetic Industry

It is estimated that approximately 500,000 [5] animals are used for cosmetic testing. Although this number is low compared to the pharmaceutical industry, many voices are raised against animal testing in the cosmetics industry due to its non-essential nature.

The FDA defines cosmetics as products applied to the human body for cleansing, beautifying, or altering appearance without affecting its structure or functions. This includes items like perfume, makeup, hair products, and moisturizers.

In cosmetics, tests on animals like rabbits and mice generally include skin and eye irritation tests.
Cosmetics are applied on animals to assess health risks.

In 1998 [6], the UK became the first country to ban animal testing in cosmetics. Over the next 20 years, countries like those in the EU followed, with the EU enforcing a full ban in 2013 (CE) n° 1223/2009.

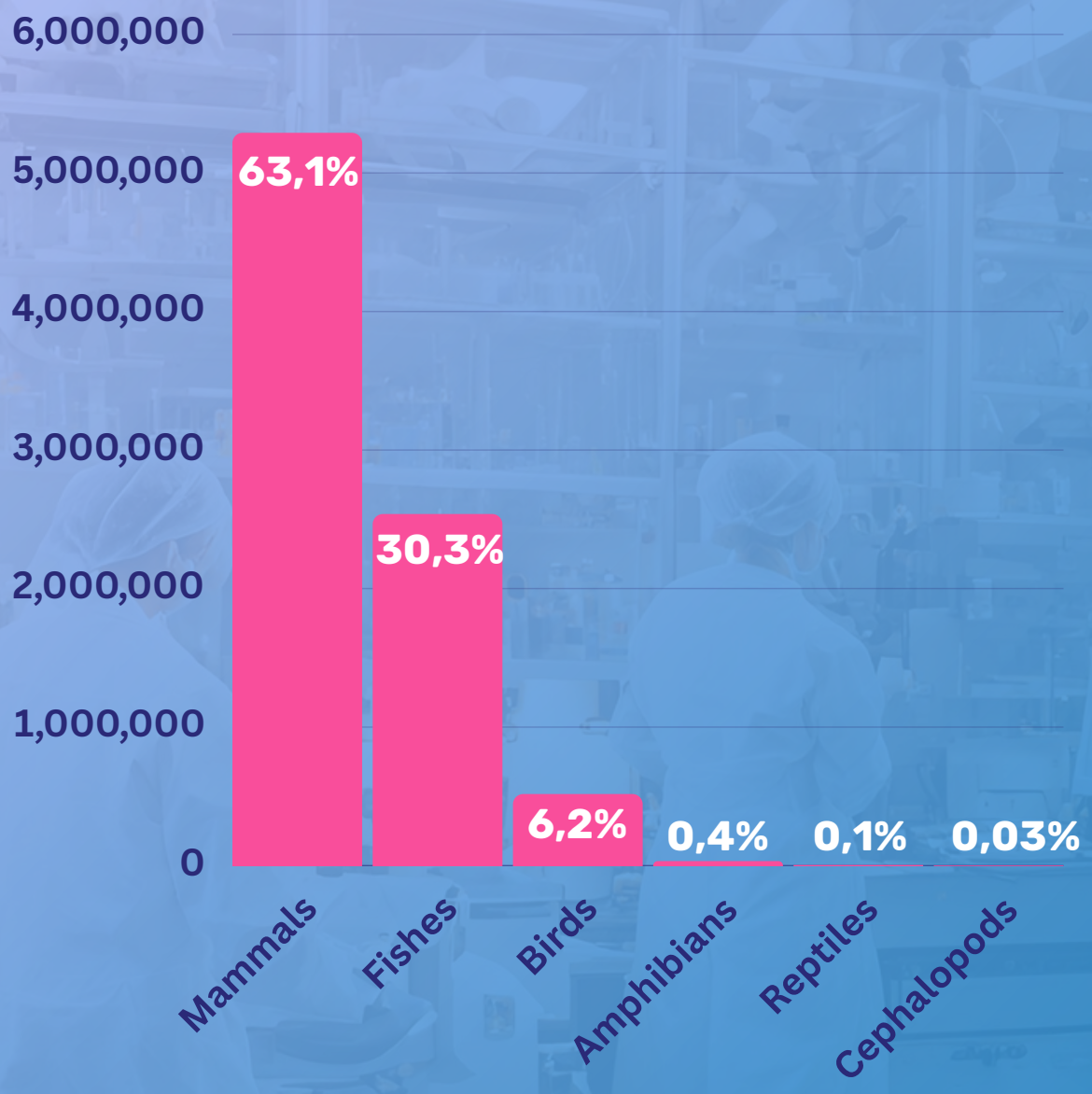
However, animal testing for cosmetics remains legal in about 80% of the world, and as of 2024, 78% of the top 50 beauty brands still use it.

ANIMAL WELFARE IN SCIENCE

How animals are being used in experiments

Factsheets – Animals testings in EU & Norway

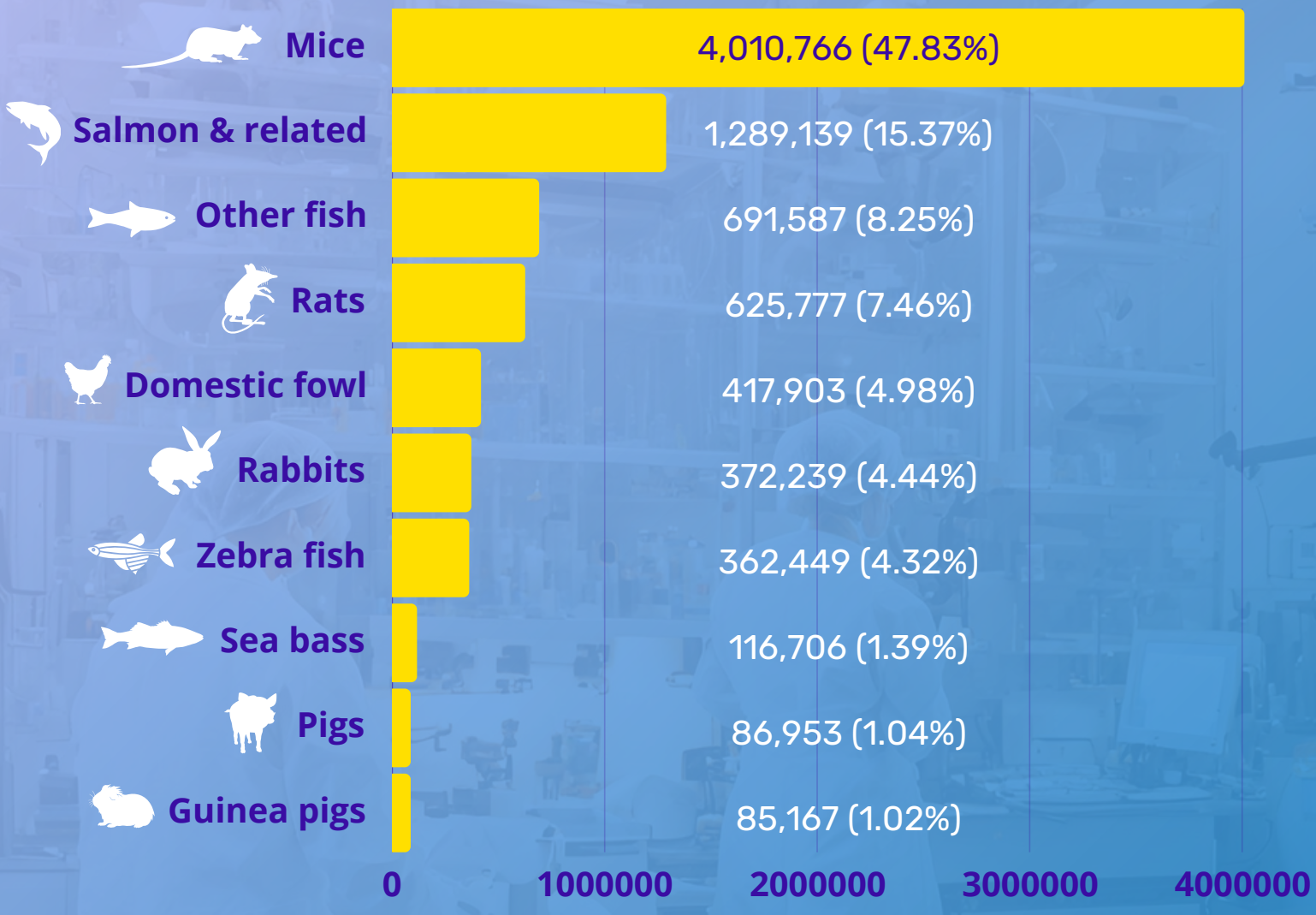
Number of animals used by category (first time use)*



***Note** Data published in July 2024 about the use of animals in the EU (excluding UK), reporting for the year of 2022. [7]

Factsheets – Animals testings in EU & Norway

Top 10 animals used for the first time in research and testing, covering 28 EU countries & Norway[7]

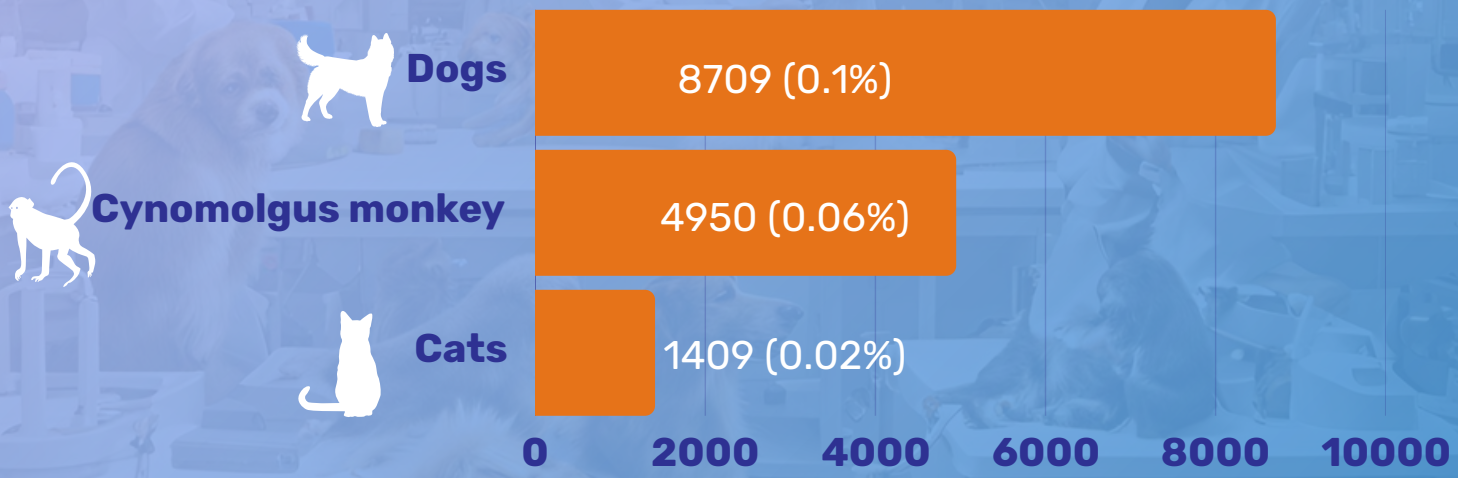


Why mice are often used in experiments?

The popularity of mice in research stems from the fact that they are genetically very similar to humans. Biological processes in mice are also, to a certain degree, comparable to similar processes in humans. Mice are easy to keep, have a short generation time and can be produced in large numbers.

Factsheets – Animals testings in EU & Norway

Data on the use of animals with special protection used in experiments[7]



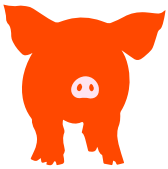
What are special protection for animals in experiments?

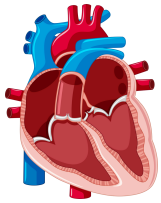
In the European Union, certain animal species receive special protection under **Directive 2010/63/EU**[8], which governs the use of animals for scientific purposes.

This directive provides additional safeguards for non-human primates, dogs, cats, and equines (horses, donkeys, and their hybrids). The use of these animals in research is subject to **stringent regulations, including specific justification requirements and adherence to the principles of Replacement, Reduction, and Refinement (the 3Rs)**. These measures aim to ensure that the use of such animals is minimized and that their welfare is prioritized in scientific research

Types of tests on Animal

Below are some examples of why specific animals are used in science. These examples cover the most specific applications but of course, animals can also be used for other purposes than these:

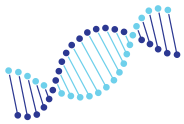




Pigs : For cardiac diseases and organ-transplant

Pigs' anatomy and physiology closely resemble humans, especially in heart structure and function. Pacemakers or stents can be tested in pigs to assess safety and efficacy before human clinical trials.

Pigs are also increasingly used in organ transplantation research due to the anatomical and physiological similarities between pig organs and human organs. In 2022, surgeons even transplanted a genetically modified pig heart into a human patient for the first time.

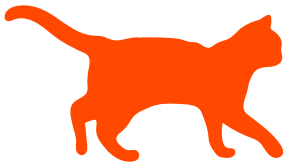


Mice and rats: Genetic manipulation and cancer exploration

Mice are genetically modifiable, making them ideal for studying the effects of drugs on specific genes associated with human conditions.

For example, it is possible to test cancer therapies using transgenic mice with mutations that mimic hereditary breast cancer in humans.

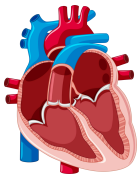
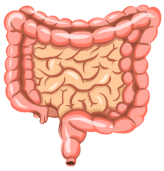
Mice are also used for Patient-Derived Xenografts (PDX). This involves grafting human tumor tissue into animals to study responses to treatment.




Cats : For Neurology

Cats have a well-developed and complex visual and auditory system, which makes them an ideal model for studying sensory processing and neurological diseases.

Cats are used to study epilepsy and other neurological disorders, as their brains are particularly suited to testing treatments for conditions like feline epilepsy, which shares similarities with certain human epileptic syndromes.

Dogs : Cardiac and gastrointestinal pharmacology

Dogs have a heart anatomy and cardiac conduction system that closely resemble humans, especially regarding heart rate and rhythm. Dogs' gastric and intestinal physiology is similar to humans in terms of pH, motility, and enzymatic breakdown.



Monkeys : for vaccine and complex human diseases

Non-human primates have a high degree of genetic and physiological similarity to humans, which has made them central to testing drugs targeting immune responses.

For example, they are used to assess the efficacy of HIV vaccines by studying viral replication and immune system dynamics in rhesus macaques.

Fishes (e.g., Zebrafish) : Environment toxicity

Zebrafish embryos are transparent, allowing researchers to observe the real-time effects of drugs on organ development and function.

Example: Screening for cardiotoxic effects of potential drugs by monitoring heart rate and morphology in zebrafish embryos.

Cephalopods (e.g., Octopus) : neuroscience and cognition

Cephalopods' unique nervous system and problem-solving abilities make them valuable for understanding the effects of neuroactive compounds. For example, we can test the impact of psychoactive substances like serotonin modulators on learning and memory in octopuses.

Rabbits: Ophthalmic drug testing

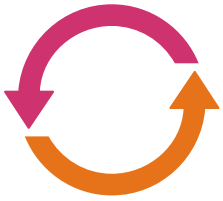
Rabbits' eyes are used for testing ocular drugs due to similarities to human eyes in tear film and corneal structure.

Example: Evaluating the safety of glaucoma eye drops to detect irritation or corneal damage.

What happens to animals after their life in the lab?

Animals are typically sacrified after experiments to examine tissues and organs for research outcomes.[9]

Some animals are intentionally killed during experiments, such as in the LD50 (lethal dose 50%) [10] test, which measures the dose of a substance that causes death in 50% of the animals tested. This test is commonly done on species like mice, rats, quail, and fish to evaluate the toxicity of substances like pesticides.



Some animals are used in multiple experiments over many years, depending on local ethical and animal welfare guidelines.

Occasionally, the animals are put up for adoption, however, this solution comes with challenges: some lab animals are not appropriate to keep as pets. Even traditional pet animals, such as dogs and cats, can have different needs as a result of living in a laboratory. These animals are used to being surrounded by fellow animals, as well as receiving attention from people throughout the day. As a result, they may not be suited to life as a house pet (particularly if an only pet, or where it will be left alone in the daytime)

The aftermath of animal testing does not stop at the tested animal. It extends to the surplus amount of animals produced but not used for research. In 2017, **12.6 million surplus animals were killed** [11] in the EU, compared to 9.3 million animals that were used for research and testing in the EU in the same year.
There's an explanation for this high amount of surplus, such as **overbreeding**, need for **specific genetic traits** and occasionally, to meet **time-sensitive research needs**, suppliers or institutions could maintain large stocks of animals.

The business of animal testing

$7 BILLION

$7 billion is the estimated annual revenue of all companies producing animals for scientific experimentation.

The major actor, headquartered in the US reported revenues of approximately $4.5 billion.

Following competitors in the US, Asia, and Europe report revenues between $50 and $500 million. Bringing total revenues of animal producers to around $7 billion [2].

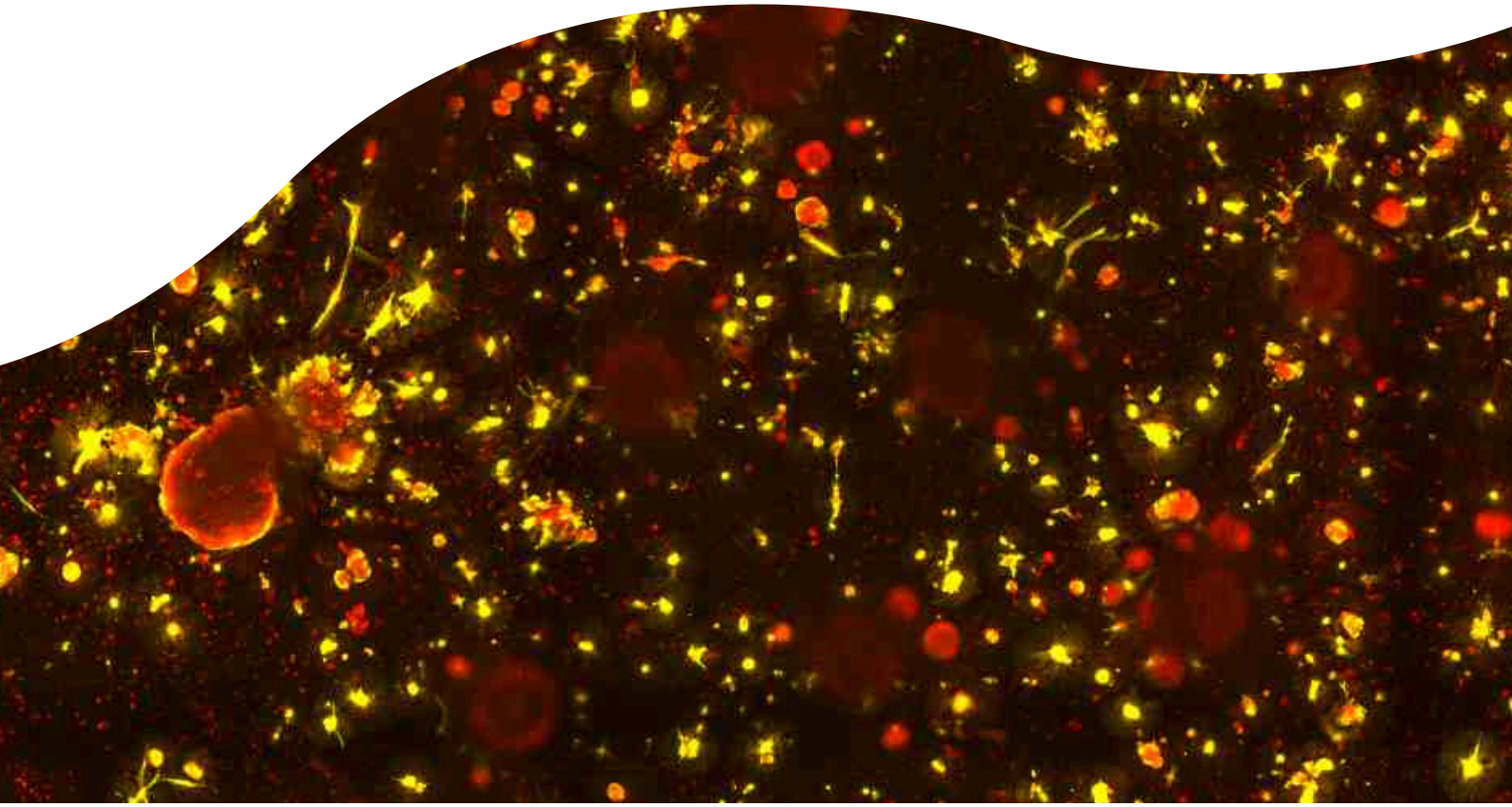
These companies provide around 90% of animals used in science. Animals can also come directly from breeding inside research facilities or from occasional suppliers in agronomic farms.

The main activity of animal suppliers is to breed animals for scientific experimentation. It includes genetically engineered animals designed to study specific diseases. One of their missions is also to store genetic material for the future.

Most of these companies also provide research services for the pharmaceutical, biotechnology, and academic sectors. This is why the total revenue of $7 billion is not solely correlated with animal production.

ALTERNATIVES TO ANIMAL TESTING

How the shift is driving the industry and the rising of alternatives to animal testing



What's the failure rate of tests on animals?

92.1%

of drugs tested on animals FAIL on humans [12]

Studies have found significant **inefficiencies in drug testing when using animal models** due to species-specific biological differences. These discrepancies often result in animal models failing to predict human toxicity accurately, leading to high clinical trial failure rates.

The LOA (Likelihood of Approval) from Phase I of preclinical trials reported a failure rate of **92.1% across all diseases**. This failure rate was even higher for **oncology diseases, reaching 94.7%** [12].

Furthermore, the use of animals in drug development is linked to substantial financial losses, with pharmaceutical companies losing billions annually—around **75% of drugs** [13] that appear safe in animal models **fail in human trials**, translating to significant sunk costs in R&D investments.

According to McKinsey in 2019, the leading 20 pharmaceutical companies collectively invest around **$60 billion annually** [14] in drug development. The average cost to bring a single drug to market, including the expenses associated with failed drugs, has risen to approximately **$2.6 billion**—an increase of 140% over the past decade. The pharmaceutical industry could encounter heavy financial loss due to the limitations of current methods, thus, highlighting the importance of developing cost-effective and more accurate alternatives to complement animal models.

3RS

3Rs - **Replacement, Reduction, and Refinement**— are principles aimed at minimizing animal use and enhancing welfare in research.

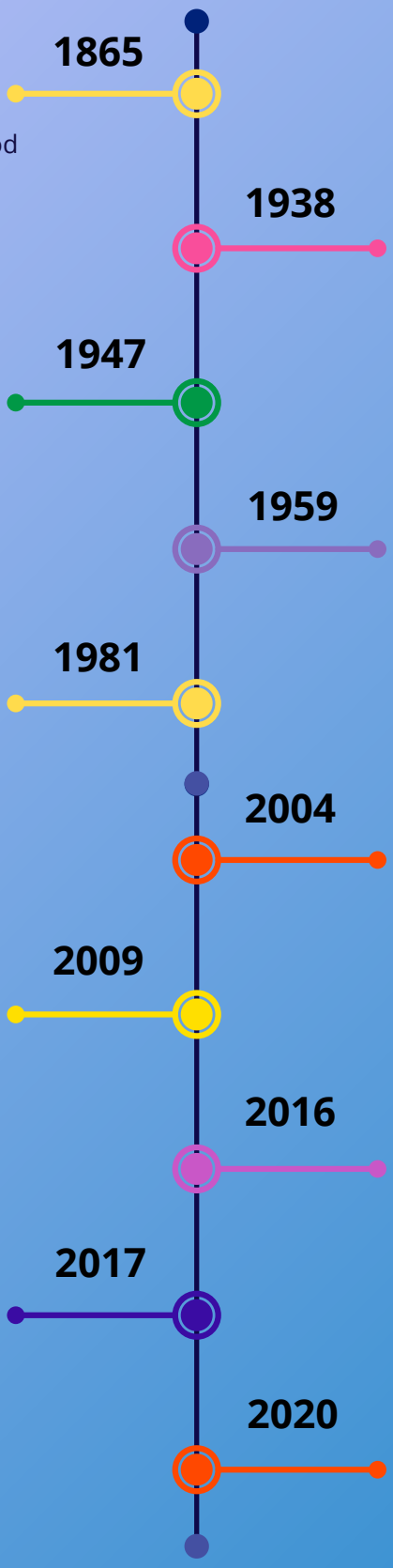
Established by Russell and Burch in 1959 [15], they guide ethical animal research practices. “Replacement” seeks alternatives to animal models, “Reduction” aims to use fewer animals for reliable results, and “Refinement” minimizes pain and stress through improved techniques.

Widely adopted in policies, such as the EU Directive 2010/63, the 3Rs remain central to humane science, with the “Refinement” factor that is considered especially impactful for directly improving animal welfare and data quality.

Knowing that animal models could be costly, time-consuming, and often come with a high rate of failure, with raising questions around ethics and animal well-being in animal testing, more and more alternatives to animal models are introduced in the recent decade, that potentially can be standardized and adopted to transform the process of preclinical trial and drug testing,

This section will review the evolution of legislation changes with important milestones that are driving the changes in scientific research, and bring to light the top 6 alternative solutions to animal testing.

The evolution milestones



1865

An introduction to the study of experimental medicine - Claude Bernard: Bernard [16] outlined the key principles of the scientific method in biology. He stressed the need for controlled experiments and argued the essential of animal testing in advancing medical knowledge.

1938

Sulfanilamide Disaster and the new system of drug control: The incident happened in 1937 in the U.S [17], when a liquid formulation of a sulfa antibiotic dissolved in diethylene glycol, resulted in the deaths of > 100 adults and children. The incident resulted in passage of the 1938 U.S. Federal Food, Drug, and Cosmetic Act, mandating animal toxicity testing

1947

Nuremberg Code: established in 1947 [18] after the Doctors' Trial, redefined bioethics by addressing Nazi medical atrocities. It emphasizes voluntary consent and safeguards for human subjects in medical experiments.

1959

Principle of the 3Rs: **Russell and Burch introduced the 3Rs (Replacement, Reduction, Refinement)** [15], which became a cornerstone for the development of alternative methods to animal testing, advocating for minimizing animal use where possible.

1981

Johns Hopkins CAAT: The **Center for Alternatives to Animal Testing (CAAT)**[19] was established to promote the development of methods to replace, reduce, and refine animal testing.

2004

Launch of Tox21: The goal of Tox21 is to research, **develop, evaluate, and translate innovative tests methods** [20], including high-throughput in-vitro, that will better predict how substances may affect humans and the environment

2009

EU Cosmetics Regulation: Article 18 of **Regulation No 1223/2009 banned animal testing for cosmetic products and ingredients in the EU**, emphasizing the need for alternative methods [21].

2016

EU-ToxRisk Project:
A European initiative funded by Horizon 2020 to **advance chemical safety testing using non-animal methods** like organ-on-chip technology [22].

2017

FDA Predictive Toxicology Roadmap: The U.S. FDA outlined a plan [23] to incorporate modern, non-animal testing methods into the **regulatory process, supporting the principles of the 3Rs.**

2020

OECD Approval of Non-Animal Skin Sensitization Test: The OECD [24] **endorsed a non-animal method for assessing skin sensitization**, demonstrating a significant step in replacing traditional animal testing methods.

The evolution milestones

FDA Modernization Act 2.0: Legislation passed in the **U.S. allowing drug developers to use advanced non-animal methods**, such as cell-based assays and computer models, for drug safety testing [25].

2022

2023

ISO/TS 11796:2023: ISO/TS 11796:2023 [26] provides a framework for evaluating non-animal methods to assess skin sensitization in medical devices, promoting ethical alternatives to animal testing and improving safety in healthcare.

2023

EU Commission's Initiative to Phase Out Animal Testing: The European Commission responded to the European Citizens' Initiative, **committing to accelerating the transition towards a Europe without animal testing** by promoting non-animal scientific research [27].

2024

FDA Modernization Act 3:0: In practical terms, this Senate vote commits the FDA to expedite the review of clinical trial applications where preliminary tests have been conducted using alternatives to animal testing [28].

"THE BEST WAY TO PREDICT THE FUTURE IS TO CREATE IT"

Abraham Lincoln

Alternatives to animal testing

Organ-on-Chip / Organoids

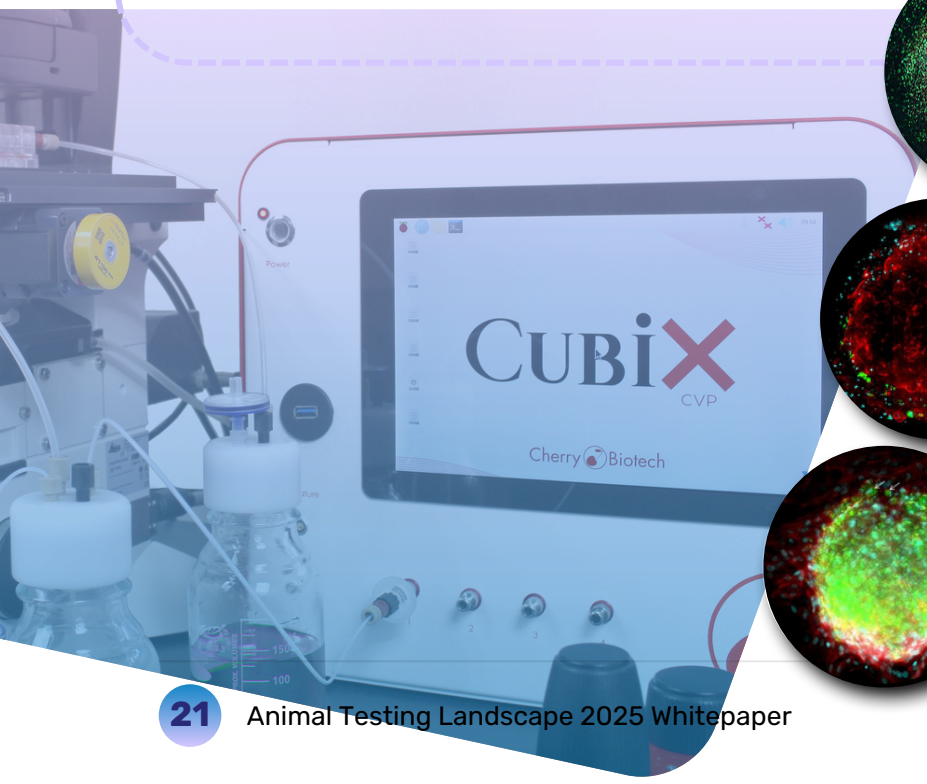
Organs-on-chips (OoCs) or Organoids are systems containing engineered or natural miniature tissues grown inside microfluidic chips. To better mimic human physiology, the chips are designed to control cell microenvironments and maintain tissue-specific functions [29].

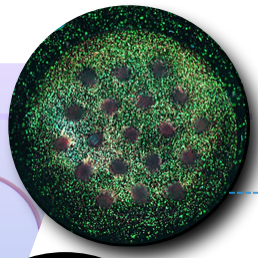
The purpose of OoCs is to imitate how human cells and organs work, including the interactions between multiple organs, to better understand complex biological processes like diseases or organ behavior.

From an engineering point of view, OoC's are microfluidic cell culture systems with controlled conditions (flow, O2, CO2 pH), that imitate the microenvironment of tissues in the human body.

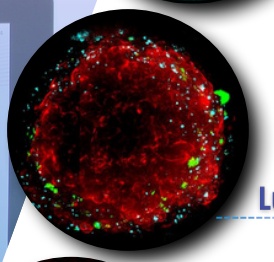
Advantages:

- Increase the precision of drug testing by using human cells in vitro instead of animal models. Study made by the Organ-on-Chip company Emulate show a 40% precision increase [30]





Breast cancer tumoroid



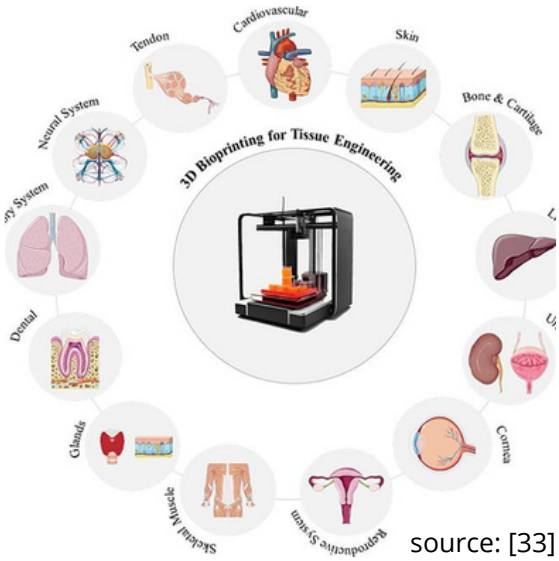
Lung cancer spheroid

Adipose tissue

Human based tissue models

Human-based tissue models are advanced structures grown in the lab to imitate how human tissues work and respond in both healthy and diseased states [31].

These models are made using cells from human donors or special stem cells called induced pluripotent stem cells (iPSCs) and are designed in 3D shapes to closely resemble real human tissues [32]. With technologies like 3D bioprinting and organoids, these models can replicate complex tissue functions, making them a powerful tool for testing drugs and studying diseases.



source: [33]

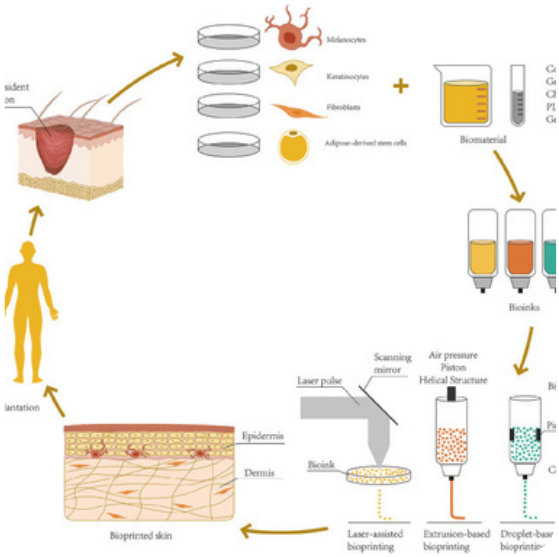
Advantages:

- More predictive insights into human responses (compared to animal models), potentially improving the success rate of clinical trials by better screening for drug efficacy and toxicity early in development

3D Bioprinting & artificial skin

3D bioprinting is an innovative technique that layers living cells and biomaterials to create structures that mimic human skin [34].

These bioprinted skin models can include multiple skin layers—epidermis, dermis, and sometimes hypodermis—with blood vessels, nerves, and other components. Used in wound healing, drug testing, and disease modeling, 3D bioprinted skin offers advantages over traditional methods, such as personalized grafts for burn victims or chronic wounds, and reduced reliance on animal testing [35].



source: [36]

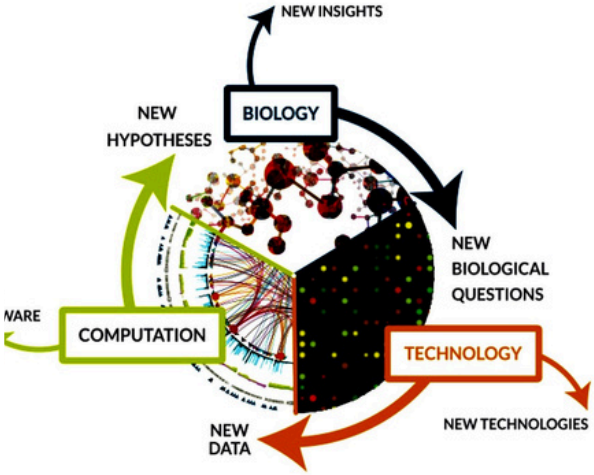
Advantages:

- Enhanced precision in replicating human skin for effective healing
- Scalability
- Faster testing of treatments, making it an advanced tool in both regenerative medicine and research

Artificial intelligence (AI) & Computational Modeling

Computational drug design uses specialized software and algorithms to predict how drug molecules behave, identify potential targets, and improve drug candidates for better treatment outcomes. This approach helps save time and reduce costs in drug research and development [37]

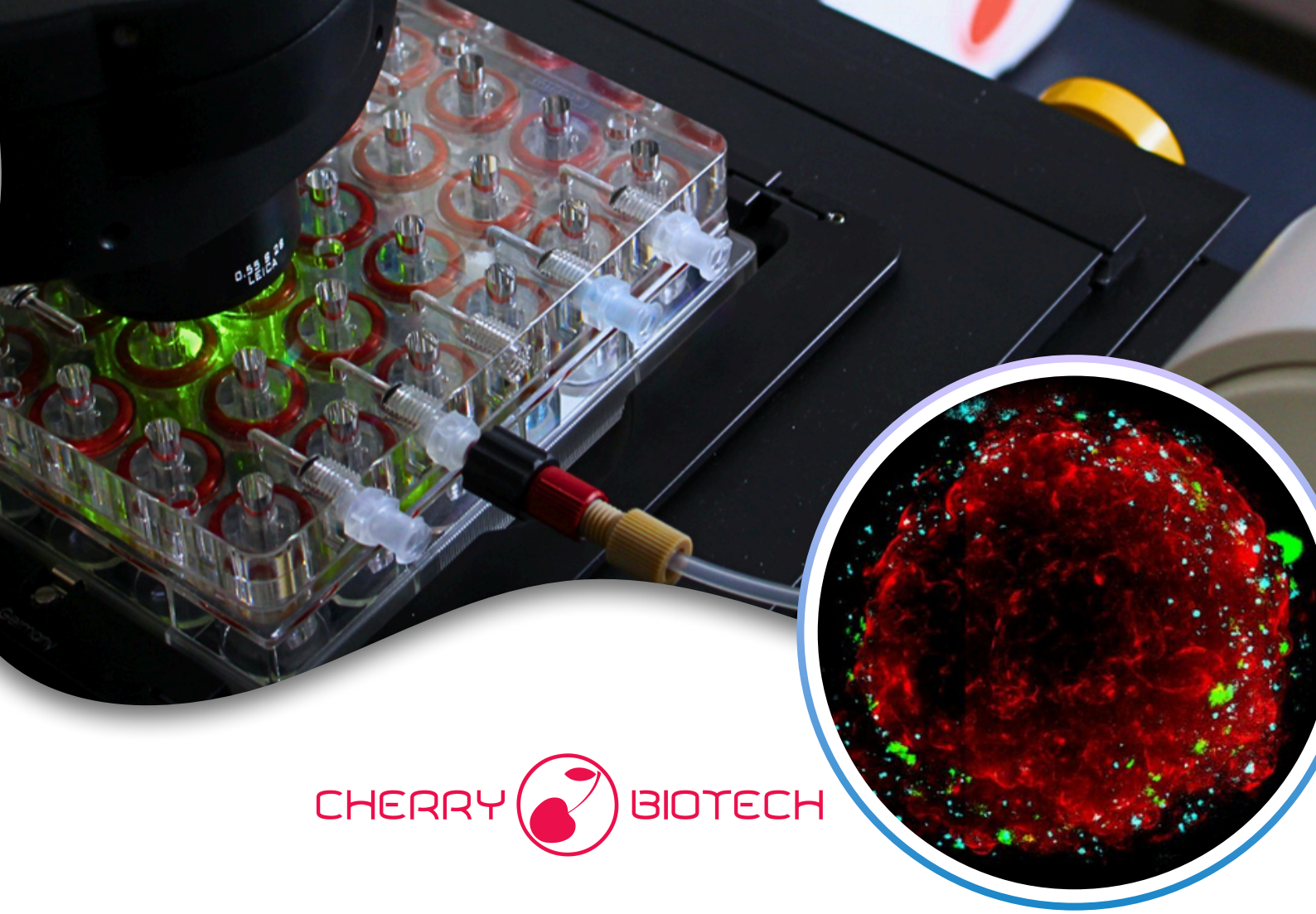
The application of AI in medicinal chemistry enhances the prediction of the efficacy and toxicity of drug candidates [38]. For example, AI-based digital patient avatars are personalized computer models that simulate the biology of a specific patient, including tumor characteristics and microenvironment. These tools make it possible to virtually test different treatments before administering them to the patient



source: [39]

Advantages:

- Better understanding of how errors arise and propagate in biomolecular modeling.
- Enable rapid, efficient design of novel compound
- Potential cost savings



CHERRY BIOTECH

In vitro human models

Cherry Biotech builds a new generation of organ-on-chips with a controlled microenvironment to replicate human physiology.
Providing scientists with accurate and ethical alternative to animals.

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