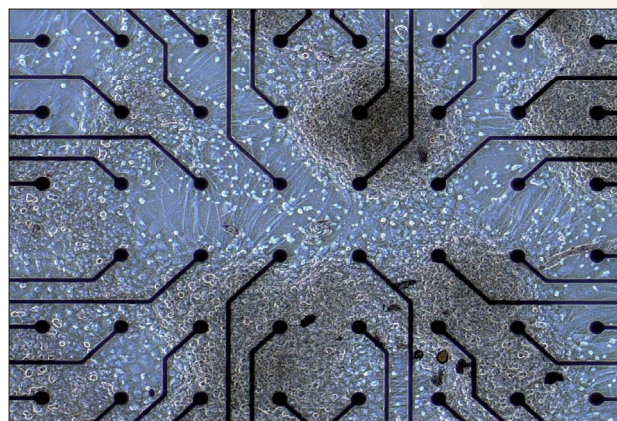


Alternative testing: SEURAT-1 achievements and way forward

How can we assess the safety of chemical ingredients without animal tests - the SEURAT-1 approach

Before a new consumer product or a medical drug can be put on the market, it must be tested to ensure that its chemical composition does not generate undesirable side-effects on human health. Most of the test methods developed in the last 50-60 years rely on animal tests, which are strongly debated because of ethical and scientific reasons.

Since decades, scientists around the world are doing research to develop test methods alternative to animal tests. The research programmes of the European Union and in some of the EU members states represent a substantial part of the global international research effort. Whereas in animal tests, laboratory animals are exposed to the to-be-tested chemical and the effects are observed and analysed, alternative methods are technology-based test methods that can be developed thanks to a better understanding of the cellular and molecular mechanisms to predict adverse effect to human health when exposed to a chemical with toxic properties. They include the following approaches:



Cortical neurons cultured on a Micro Electrode Array chip

- ➡ *In vitro testing* is based on cells originating from different species (including humans) that are cultivated (i.e. grown to obtain the required large quantities), to evaluate the effect of chemicals at the cellular level. *In vitro* tests are already applied in the safety assessment for some specific consumer products such as cosmetics but also for other chemicals, and their application can be extended by further developing the required cell technologies and test methods.
- ➡ *Bioreactors (also called “microfluidic device” or “organ on a chip”)* are tiny devices conceived to overcome the limitations of single (mono-type) cell based tests mentioned above, by combining different cell types in a miniaturised, closed system fed by a micro-fluidic circuitry. This technology has progressed considerably over the last years, but is not yet mature enough to be used in standard safety test practice, and needs further development and standardisation.
- ➡ *In silico* methods apply computer models to predict possible toxic effects of chemicals to the human body. *In silico* methods include both dynamic and kinetic modelling. Dynamic models predict a chemical's interaction within a cell or tissue, e.g. protein binding. Biokinetic models instead simulate how a chemical is absorbed, distributed, metabolised in the human body, and finally eliminated.

SEURAT-1 is one of the more recent European large scale research initiatives on alternative methods. SEURAT stands for «Safety Evaluation Ultimately Replacing Animal Testing», and the «-1» indicates that it is one step of a long term research effort needed to achieve the final goal. It is a joint effort of public research programmes and the industry (a so-called «Public-Private-Partnership») that has been co-financed by the European Union's research Framework Programme 7 (FP7) and Cosmetics Europe, representing a 5 years (2011-2015) and 50 million euros research effort. SEURAT-1 gathered over 70 European research groups from universities, public research institutes and private companies, carrying out their research work in close collaboration with the industry, regulatory bodies and related international research initiatives, with the common aim to make a substantial step forward towards the implementation of alternative test methods.

SEURAT-1 started from progress achieved by previous research projects on biological mechanisms of toxicity and the development of new technologies, in particular biotechnologies such as cell engineering, microfluidics, massive data processing, computer modelling, robotics, and more. The SEURAT-1 main achievements are described hereafter.

What has SEURAT-1 achieved to advance alternative test methods?

Proof-of-concept and stakeholder awareness

The main objective of the SEURAT-1 initiative as a whole was to demonstrate scientifically that alternative test methods are capable to predict if a given chemical has a toxic effect on human health after repeated exposure (so called «repeated dose toxicity»). SEURAT-1 provided this proof-of-concept with the help of case studies and succeeded to deliver an essential message to a large range of stakeholders. The interest in SEURAT-1 shown by EU level regulators indicate that SEURAT-1 achieved the aim to increase awareness about the potential impact of alternative test methods in safety assessment.

Integrated approaches

SEURAT-1 developed a “conceptual framework” for an integrated testing strategy. A practical implementation of this was illustrated through the setup of an Educational Guided Tour shown for the first time at the SEURAT-1 symposium on 4 December 2015. You can download the associated booklet on the SEURAT-1 website in the following link: http://www.seurat-1.eu/media/Guided_Tour_Booklet.pdf

Long term strategy for alternative testing research

SEURAT-1 defined a long term strategy for alternative testing research built on “mode-of-action”, i.e. the understanding of the mechanisms by which certain chemicals produce an effect on a living organism, a strategy that goes far beyond the duration of a single project and can hence be used as reference for other research projects.

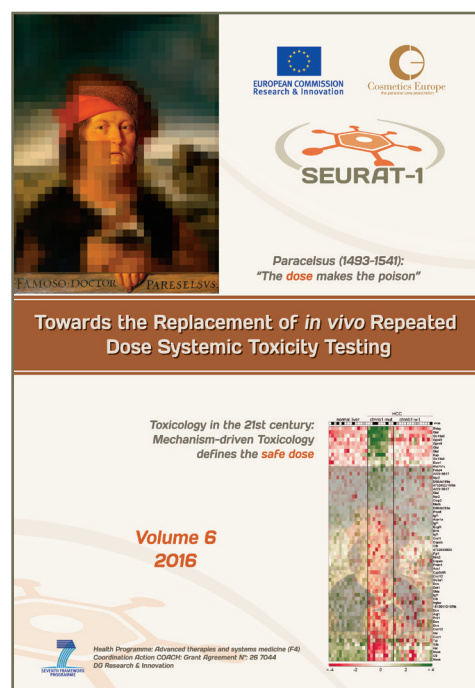
Significant progress in knowledge, methods and technology

SEURAT-1 was conceived as a cluster of several complementary research projects, each focusing on a specific research and development domain that is key for the implementation of alternative testing solutions. In each of the alternative test approaches mentioned on the previous page (*in vitro*, *bioreactors*, *in silico*) the SEURAT-1 projects made significant progress in the knowledge and technologies required to:

- ➡ produce cells with the characteristics, quality and quantity required for alternative test methods,
- ➡ implement a “liver-on-a-chip”, i.e. a bioreactor equipped with liver-specific cells (a liver-simulating device) suitable for long-term toxicity testing,
- ➡ create an integrated suite of computational tools to predict toxicity in human,
- ➡ identify biomarkers, i.e. measurable biological signs indicating that a given chemical has a toxic effect in the human.

SEURAT-1 also demonstrated the application of the developed tools and concept in three case studies:

- ➡ The “read-across” case study, where the safety data available for one data-rich chemical is used to predict safety for another data-poor chemical, considered to be similar based on the chemical structure and biological behaviour. This application scenario can already reach regulatory acceptance, which was shown by the implementation of the ECHA Read-Across Assessment Framework discussed at the Scientific Topical Workshop at ECHA in April 2016.
- ➡ The “*ab initio*” case study, illustrating proof of concept of how risk assessment for a cosmetic ingredient might be carried out without animal testing by building up evidence based on a logic workflow from new approach data.
- ➡ The “Threshold of Toxicological Concern” (TCC) approach for dermal exposure, more relevant to cosmetic products, which was previously developed for oral exposure. TTC establishes a level of exposure below which there would be no appreciable risk of the chemical to human health.



Volume 6 of the SEURAT-1 Annual Book series
You can download the Annual Book from
the SEURAT-1 public website:
see box on the last page.

Legacy to support the continuation of the research effort

As its name indicates, SEURAT-1 has been conceived with the idea in mind that a continued longer term research effort is needed to reach the final goal, i.e. the replacement of the animal tests by alternative test solutions. The entire research work of SEURAT-1 has been documented in a series of **Annual Reports** that was published in 6 volumes in printed and electronic form that are available on www.seurat-1.eu. A **data warehouse** has been setup and will be maintained after the termination of SEURAT-1, so that the information and data generated in this research programme remains available for the following research initiatives. Furthermore, a **methods and tools catalogue** has been edited in particular for the industrial users, collecting methods and tools developed in the SEURAT-1 framework, which is available through DB-ALM, the European Commission database on alternative methods (<https://ecvam-dbalm.jrc.ec.europa.eu/>).

Current state of play and the way forward

The research work done by SEURAT-1 and the many other projects and initiatives in Europe and world-wide has allowed to make an important step towards the development of alternative testing methods. As mentioned above, some alternative methods are currently already applied in regulatory safety testing practice. However, the current level of scientific knowledge and technology is not advanced enough to propose alternative solutions for the majority of the safety assessment tests, and especially the more complex ones. Some examples of the remaining important gaps that need to be addressed are:

Understanding of toxicological cellular and molecular mechanisms

Further research is needed to study toxicity mechanisms on the multiple levels of the biological organisation, including genes, proteins, pathways, and cell/organ function. For example, in repeated dose toxicity the underlying processes include effects at different biological levels and time scales that are extremely complex. *In vitro* methods have generally been developed to predict effects in specific target organs, without considering how the information generated can be used to predict the complex *in vivo* interactions between different cell types and tissue systems leading to toxicity. Part of the solution can be further development and application of biokinetic models.

Integrated approaches

There is a need to fit *in vitro* and computational methods together in integrated approaches and testing strategies. A clear limitation of alternative test methods in the context of regulatory safety assessment is the lack of tools to address «toxicokinetics», in particular the effect of metabolism of compounds in the body, i.e. the way a chemical substance behaves during the metabolic breakdown process which can change its impact, and notably its toxic effects.

Production of robust solutions ready for validation

Once integrated approaches of *in vitro* and computational tools have been developed and demonstrated to work reliably in laboratory conditions, robust solutions need to be developed that can be deployed and used in routine practice and conditions. This includes the standardisation and reproducibility of the test methods as well as the capability of up-scaling the developed methods, i.e. the possibility to use them on a large scale by implementing highly automated «high-throughput» industrialised processes.

Validation of the reliability and relevance of alternative test methods and regulatory acceptance

The validation process generates and/or assesses empirical information on reliability and relevance of a test method/approach under standardised and controlled conditions. Therefore, this process is generally accepted to facilitate and/or accelerate the international (regulatory) acceptance of alternative test methods/approaches. EURL ECVAM, the European Union Reference Laboratory for alternatives to animal testing, is coordinating validation within the European Union. The successful methods might then reach international acceptance through OECD Guidelines for the Testing of Chemicals.



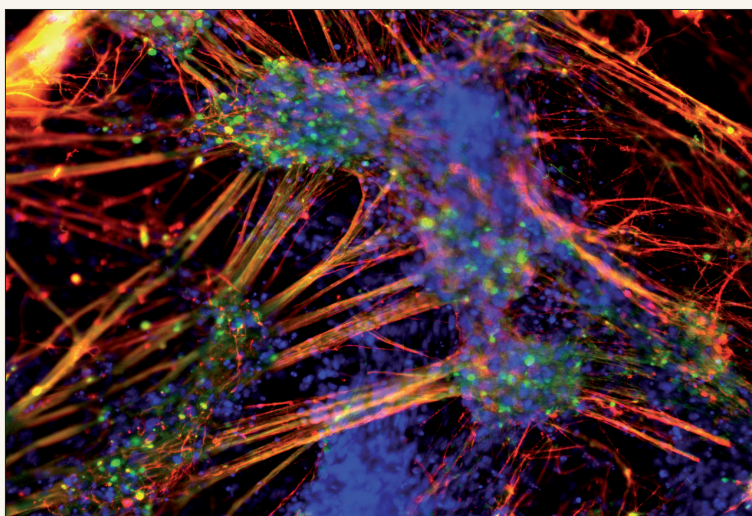
Hamilton Robot at the ECVAM High Throughput Screening facility

The need for a continued research effort

SEURAT-1 is now completed, but the research effort on alternative test methods is continued by other initiatives. The main SEURAT-1 follow-up research project in Europe is **EU-ToxRisk**, a large scale («Flagship») research project supported with 30 million euros of funding by the European Union's Horizon 2020 programme for 6 years (2016-2020) in the continuity of the SEURAT-1 initiative. In addition to repeated dose toxicity, EU-ToxRisk also looks into developmental and reproductive toxicity, i.e. the impact of a toxic ingredient on the embryo and the reproductive capacity of human. There are many other European ongoing research projects in the area such as MIP-DILI, HeCaToS, EuroMix, EDC-MixRisk, eTOX, to mention a few.

On the **EU member state level**, alternative testing research is supported by national research programmes and initiatives. The situation is quite different from one country to another and some of the member states have dedicated funding for alternative methods research. Depending on the country there is a wide spectrum of supporting measures and project types ranging from basic research to final development phases to help in particular SMEs to enter the rapidly developing market of alternative testing products and services.

At the international level, the major initiative supporting the development of alternative testing approaches is the US Tox21 programme, a federal collaboration among EPA, NIH and FDA. Tox21 mainly uses high-throughput technologies to screen thousands of chemicals for potential toxicity, use screening data to predict the potential toxicity of chemicals and develop a cost-effective approach for prioritising the thousands of chemicals that need toxicity testing.



Neuronal connections and cellular nuclei in neurons derived from human stem cells

The needs in resources for leading alternative methods research and innovation forward are huge, and there are still many years to go before alternative methods will dominate safety testing in regulatory practice. All actors in alternative methods research need to continue their efforts in this field with a long term vision and strengthen the collaboration at national, European and international level to optimise the use of the resources. Alternative testing solutions are not only a response to ethical concerns, but are also expected to address currently unsolved problems in the safety testing domain by significantly improving the accuracy, scalability and cost-efficiency of safety tests, and represent a huge business potential for European companies.

You can download the SEURAT-1 Annual Reports by following this link
<http://www.seurat-1.eu/pages/library/seurat-1-annual-reports.php>



For more information about
SEURAT-1 and the related research work:
www.seurat-1.eu