

# WHITE BOOK

A Guide Book for More Effective Transfer of  
Alternative Methods from the Research Stage  
to Applicable Tests for Product Safety.

forinvitox

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# Introduction

Research on in vitro replacement tests by scientists and industry, with substantial support by the European Commission, has resulted in an important number of scientifically sound methods and new strategies. However, the transfer of these inventions to potential users has been much slower than expected, mainly due to difficulties encountered in the transferability, official approval as well as production of test kits under conditions of regulatory requirements. The present gap between inventions and potential users needs to be bridged.

This White Book, the final report from the Forinvitox project, has the intention to present recommendations on how to promote the development of alternative methods at the research stage to tests that are available and applicable for the end users for the European Commission. The book is also written to be a useful guide for inventors, developers and manufacturers of new toxicity tests as well as for those companies that test their products for consumer safety.

## **The Forinvitox Project**

The Forinvitox Project, supported by the European Commission within the sixth framework program, had the purpose to facilitate the development and increase the number of commercially available in vitro methods by identifying the most urgent testing needs and the ways to respond to them.

The German and Spanish national platforms of alternatives (REMA and SET) investigated the development stage of the methods evolved from the FP6 projects. They found a number of interesting methods that need to be further transferred to become applicable for the end users.

The In vitro Testing Industrial Platform, IVTIP, made an analysis among their members of the needs of the users. There is a need for more mechanistic and physiologically relevant models. It is also important that the methods are robust and reproducible.

Expertrådet made an inventory of the manufacturers of in vitro tests. It showed that their major customers are from the pharmaceutical industry. A serious problem for the technology transfer of the methods is the lack of intra and interlaboratory reproducibility.

The reports were summarized and discussed with experts representing the stakeholder groups. This round table meeting, arranged by Silverdal Science Park, came to the conclusion that the most important issues to improve the technology transfer of in vitro tests are to:

- Bridge the gap between inventors and end users
- Improve the development process of in vitro models
- Invent and use instruments to facilitate the transfer

With the Expert meeting in mind, the partners of Forinvitox drew up the outlines for the Forum Event, an experiment in facilitating the transfer of alternative tests. A market place for inventions, applications and products was arranged for the first time in Europe. Aspects highlighted at the Expert meeting were presented in short lectures and then discussed with all participants.

The Forum Event was a joint event of the EU-projects Forinvitox and Invitopharma and IVTIP and organised by Silverdal Science Park. 30 models with commercial potential were presented at the Forum. 17 of them were presented orally and then discussed with focus on commercial applicability, first by a panel and then by the whole audience. There was also plenty of time for spontaneous discussions and meetings during the Market Time. To stimulate new meetings between the participants were the organisers active in matching people with similar questions or with questions and answers.

The project website has been reorganised several times to mirror the activities within the project. During the first part of the project had the manufacturers of in vitro tests opportunity to inform about their products. The website was reorganised to the workshop website before the Forum Event and now it presents the outcome from the Forum Event such as the presentations of the invited speakers and methods.

The results from the project will be summarised in this White Book that will comprise:

- A guide for the European Commission on how to promote the transfer of alternative tests to applicable products for the end users
- A guide for the inventors of alternative test in the process of transferring a research method into an applicable test
- A guide for the end users about present and upcoming methods within the area of alternative tests.

# Bottlenecks for effective technology transfer of alternative methods

The different actions within the Forinvitox project have identified the following bottlenecks in the transfer of alternative methods at the research stage to tests for commercial product safety testing.

## The gap between invention and commercial use

### **The inventors view**

Sometimes there are lacks of interest by academic researchers to commercialise the methods they have developed. Different expectations and difficulties makes it sometimes difficult to come to an agreement between the inventor and the company that is going to do the exploitation.

### **The manufacturers view**

It is important to speed up the validation procedures and to increase co-operations between producers, users and researchers of alternative toxicity tests to meet the future demands on true replacements for in vivo tests.

### **Comments from the Expert meeting**

The gap comprises optimisation, validation, implementation and development to a product and there is a lack in financing as well as organisations that could bridge the gap. It is not possible to cover every part in the implementation process in one project. As an

example, the role for a scientist could cover three years of research followed by two years of development within another specialised group, e.g. a CRO. Working with the different steps of “the gap” needs to be upgraded and more attractive.

## Quality

### **The inventors view**

There are often large inter- and intralaboratory variations even though the same standard protocol is used. There is a need for training activities.

### **The view of the end users**

Companies pointed out that they have a special interest in the quality of the results obtained even while they are considering them as qualitative indicators. Since the models used in the companies are matching with their expectations, the final users are willing to obtain more relevant models with increasing relevancy about the results, a good quality of product (GMP like production and batch), the robustness of the results and reproducibility. In other words, it seems that the users have defined the strengths and limitations of the testing models they use and established a system to apply them in their R&D process.

### **The manufacturers view**

The largest problem reported when a research method is transferred into a commercial assay is the lack of intra- and interlaboratory reproducibility.

The second largest problem is the need of common routines, SOPs, and result calculations but also to the problem of different knowledge and experience in the laboratories is important. Other problems and bottlenecks are the complexity of equipment (instruments and models/assays), the lack of clear positive and negative reference compounds, validation procedures, and the lack of correctly registered historical data (traceability of data). Training of personnel on how to use the assay/model, and the quality controls at the laboratories and production facilities need to be improved.

### **Comments from the Expert meeting**

Reproducibility is a crucial point in technology transfer and a change in mentality within academia concerning the importance of reproducibility is badly needed. However, there are different criteria for reproducibility dependent on the application of the method, e.g. screening, hazard identification or risk assessment.

The experts representing the end users pointed out that GLP or other quality systems are an absolute must for the use of methods in commercial application. They would not work with CROs or test kits produced without GLP, GMP or any other suitable quality system. This concerns larger companies as well as SMEs. The academic scientists have to think about quality and reproducibility already at the beginning of method development.

Usually there is a need of simplifying and increasing the robustness of a new method during the development procedure. However, the academic scientists should not regard this transformation as a failure. It is also important that the end users do not inhibit the creativity during the research phase. One should also keep in mind that there are research models and test models that have to answer different questions and therefore are not easily replaceable.

Standardisation and clear guidelines for prevalidation and validation including the writing of SOPs would certainly increase the reproducibility and facilitate the procedure of transfer. This also applies for clear criteria for the submission of new methods into the OECD process for acceptance in guidelines. Increased transparency of the process of validation and submission for the OECD process for acceptance as well as a clear timeline for the procedure are needed. If the test methods shall be available for the end users in reasonable time it is important to speed up these processes. The process works better for drug testing, since they have extensive guidelines on what is needed for the application to the Medical Authorities.

Standardisation and customers' training as well as proper and usable SOPs should be provided by the provider/manufacturer of a test method to secure good quality.

## Acceptance

### **The inventors view**

The validation process is the most important way to get the methods accepted. This process needs to be speeded up. The acceptance would probably increase by considering the mechanistic relevance of the methods under development as well as an early involvement of regulators.

### **The view of the end users**

Most frequently, it was indicated that a single end point (like cytotoxicity) is used. Although it is recognised that cytotoxicity is a weak endpoint, it is considered as sufficient for the expectations assigned to the assay. Nevertheless, the results obtained with in vitro methods have a limited impact on the corporate final decision mainly due to the lack of clear and unambiguous guidelines. An additional important point is that the users are waiting for specific guideline(s) on how to implement their battery of assays

### **The manufacturers view**

The lack of regulatory and scientific acceptance for alternative toxicity tests, as well as the inherent conservatism of regulatory processes is a draw back for commercialising alternative methods. Proper guidelines for validation, which includes the need of policies and standards to validate assays, definition of materials for validation studies, capacities for e.g. statistical support, and quicker procedures, for example. Increased efforts to compare and validate in vitro data with in vivo data would increase the acceptance for alternative toxicity tests.

### **Comments from the Expert meeting**

There is a climate of change concerning application of in vitro test methods. Europe is keen to speed up the process of acceptance. To accept a new test model on the European level you need a majority but within OECD you require consensus. US authorities are more flexible in bringing in new “alternatives” in the meaning to exchange an animal method against an in vitro method. There is an international cooperation for harmonization. Common criteria would be helpful for the acceptance of in vitro methods.

Legal acceptance would probably increase if the stakeholders - the legal authorities, industry, CROs and academia - cooperate from an early stage during the development of a new test method. There should be a continuous communication about new methods with regulators. In vitro methods can give more information on what mechanisms are involved in the toxicological response compared to the established animal tests. The demands of quality of in vitro methods today are much higher than when the existing in vivo methods were established. It is important that those who develop a method are clear in their communication of how a new method works. Science comes closer to regulatory regulations every year.

In order to facilitate scientific acceptance (as well as making it more attractive for scientists to spend time in developing new methods), new and improved alternative methods should be published in peer review journals. The journal “Toxicology in Vitro” has a section for new methods and some other journals also publish articles about methods. Considering the issue of publishing versus patenting there is no problem to publish the results the day after submitting a patent application.

# Technology transfer

## **The inventors view**

There are difficulties for academic researchers to finance the commercialisation. Grants for technology transfer are needed.

## **The manufacturers view**

There are difficulties to finance the commercialisation as both test optimization and validation processes are very costly and quite hard to get funded. Also material and equipment can be expensive.

## **Comments from the Expert meeting**

The term “Technology transfer” includes several different components and meanings. In the following discussion it is defined as the process of implementation of a method which will most often result in a commercial product.

### *Technology transfer matters concerning FP-projects*

Today there is a paradox in the work on disseminating results from the FP-projects as you have to use the grants before the project ends. Usually the researchers do not get any results to disseminate until the project has been finalised. The dissemination would be more effective if the money could still be used for , e.g., a year after finalising the project. Today, it is difficult to get hold of the outcome of FP-projects. As public money is used for funding, the results of the projects should also be public.

The management of the FP-projects is very important and needs to be a larger proportion of the project budget. The management should be designed to be compared with the management for large projects within companies. Smaller and more focused projects with

about 7-8 partners could also improve the efficiency in implementing new techniques.

The technology transfer from the FP-projects would probably also improve if there were more interactions between the FP-projects and the final users during the whole project. There should also be more discussions about technology transfer matters within the advisory boards and steering committees. The research within a project can give rise to several potential products, not only from the planned results. The process of technology transfer should start before the end of the project otherwise it is a risk to lose everything accomplished in the projects.

### *General aspects*

Development of a new method in co-operation with a customer is a possible way to finance the development/technology transfer. A crucial point is of course that the product is promising enough. The development process requires training between laboratories. The process of scaling up the production of a method takes years.

There is not just a particular step or part of the development process that should be reformed to improve the technology transfer but there are scientific, economical, commercial, technical, political as well as legal obstacles for an efficient transfer of in vitro methods today.

# Capacity

## **Comments from the Expert meeting**

To be able to analyse if the capacity in Europe can meet the testing demands in the future, e.g. REACH, more background information is needed. For example which areas of toxicity testing will be of most importance for in vitro methods in the future? The demand depends primarily on regulation aspects. It is also important to define the steps of the development process before it is possible to identify if there is insufficient capacity at any particular step. However, reliability, continuity, GLP and knowledge in how to use the new methods are always aspects of significance when estimating capacity.

It is important to keep close contact with the different organisations representing the final users of toxicity tests, e.g. IVTIP (the In Vitro Testing Industrial Platform), EPAA (the European Partnership for Alternative Approaches to Animal Testing) and Europa-Bio (the European Association for Bioindustries).

# Recommendations to the European Commission

As a result from the Forinvitox project comprising an analysis of the retrieved data, the “Summary Report”, round table discussion with the stakeholders at the Forinvitox Expert meeting and the innovation market the Forum event are the following actions proposed to promote and improve the transfer of alternative methods from the early stage of research innovations to commercial use by the end users.

The following issues were identified as the most important to improve the technology transfer of in vitro tests.

- Identification of the gap between the inventors and the final users.
- Bridging the gap
- The development process
- Instruments to facilitate the transfer

## Identification of the gap between the inventors and the final users

The discussions during the Forinvitox Expert Meeting came to the conclusion that the nature of the gap between the inventors and the final users is:

- Intellectual
- Cultural
- Financial
- Legal (concerning dissemination of results)

## Bridging the gap

To be able to bridge the identified gap there is a need of:

- Financial bridges like EC or national calls specified for verification of methods as well as other steps of the transfer process. Collaboration with final users and CROs.
- Intellectual and cultural bridges like brokers, meetings and networking. Forinvitox could be one of the bridges.
- Specified calls for optimisation, validating, implementation and technology transfer for academia as well as companies that could be organized in cooperation between national funds and European Commission.
- Criteria for the different steps of developing a new method.
- Closer collaboration between the stakeholders, the academic scientists, industry (manufacturers and final users) and legal authorities at an early stage of the development.
- Stimulating and facilitating the discussion between inventors and producers and users with technology transfer knowledge.
- Intellectual and cultural bridges like brokers, meetings and networking.
- Facilitating the matching of inventions with applications.

## **The development process**

The following parts are essential for the transformation of an invention to a commercially applicable test method:

- Transformation to a less complex method
- Robustness
- GLP
- Validation
- Different competences for the different steps of the process
- Early involvement of regulators

## **Suggestions and instruments to facilitate the transfer**

- Clear guidance and criteria for validation
- Showing good and bad examples
- Cookery-book for the steps of the transfer process
- Matching inventions with applications
- Presentation of new methods from a very immature stage to more mature methods for producers of test methods and end users. The Forum event and the ForinviTox web site could work as useful platforms.
- Training and/or easily accessible advice on technology transfer and IP matters for scientists

## **FP-projects**

- Increased investment in the project management by training, recruitment and a larger portion of the project budget.
- Dissemination of the results after finalising the project.

## **Suggestions for increased quality**

- Training courses in quality control and statistics at basic academic level as well as advice in quality control at different levels.
- Peer reviewers should be informed in statistics and GLP.
- Definition and set up of relevant positive control for each model.
- Standardisation and clear guidelines for prevalidation and validation.
- Clear criteria for submission of new methods in the OECD process for acceptance.
- Clear timeline and increased transparency of the process of validation and submission for the OECD process for acceptance.

## **Suggestions for increased acceptance**

It is important to communicate about new methods and new techniques to relevant groups outside the scientific community to increase the acceptance of in vitro methods.

## **Identification of needs**

Instead of defining any particular toxicity area a general need to develop and define more robust and less complex methods easily applicable for the end users. The efforts should be focussing on the development of strategies more than on separate methods. Discussing toxic mechanisms should have priority over end points in animal testing.

# Conclusions

## **In relation to next calls and next 8FPs**

A larger fraction of the budget should be dedicated to dissemination and technology transfer, and it should be made obligatory to project management to actively pursue (and demonstrate) dissemination and technology transfer. IVTIP has suggested a few years ago to dedicated 12.5%.

## **In relation to positive actions for communication/interactions for increased transfer of technology and applicability and for alternative tests**

A newly developed industrially applicable testing method should at least comply with the following set of criteria:

- Capturing key events of the in vivo mechanism of action
- Be relevant to the human exposure
- Reproducible and reliable
- Readily available
- User and implementation friendly
- Validated (scientifically or formal) for a specific purpose
- Economically viable
- Cost effective

## **To do legal actions for increasing acceptability for in house use/validation and for regulatory acceptability**

# The Inventors Offer

The German and Spanish national platforms of alternatives (SET and REMA) investigated the development stage of the methods evolved from the FP projects. Because of limited access to information about the FP5 projects and that the FP6 projects are considering transfer of technology in their objectives the investigation was focused on the FP6 projects.

## Project **ACUTETOX**

Contact: Dr. Cecilia Clemedson

<b>Test models included</b>	Cytotoxicity	The Caco-2 model	Colony forming unit-granulocyte/ macrophage (CFU-GM) assays
<b>Methods with commercial potential</b>	Already used commercially	Already used commercially	Yes
<b>Endpoints</b>	Acute toxicity	Subacute and subchronic	Toxicokinetics and metabolism
<b>Expected field of application</b>			
<b>Stage of development</b>	Basic research on mechanistic end points	Optimization method	Pre-validating
<b>Expected regulatory acceptance</b>	Yes		
<b>Expected interest from the market</b>	All end users and manufacturers		

	The whole blood assay	LLC-PK1/TER model for nephrotoxicity	Different assays for testing neurotoxicity	Cytomic	Neural network
	Yes	Yes	Yes		
	Ecotoxicology	Blood brain barrier	Nephrotoxicity	Hepatotoxicity	

Project **SENSITIV**

Contact: Erwin L Roggen

<b>Test models included</b>	Genomic	Proteomic	Enzyme-receptors
	Cell differentiation	Ex vivo tissues/lung	Artificial skin
<b>Methods with commercial potential</b>	Properly evaluated test systems		
<b>Endpoints</b>	Lung sensitisation		
<b>Expected field of application</b>	Chemicals	Cosmetics and hygiene products	Pharmaceuticals
<b>Stage of development</b>	Basic research on mechanistic end point	Optimization method	In-house optimized
<b>Expected regulatory acceptance</b>	EC-ECVAM		
<b>Expected interest from the market</b>	Specific end users are already applying for testing.	End users are interested and supporting	We know users which are interested

Cell functions	Cell proliferation
Ex vivo skin preparations	
A producer technology company is already commercialising	A producer technology company is interested in commercialising

Project **VITROCELLOMICS**

Contact: Prof. Karl-Fredrik Mandenius

<b>Test models included</b>	Cell functions	Toxicokinetic model	
<b>Methods with commercial potential</b>			
<b>Endpoints</b>	Toxicokinetics		
<b>Expected field of application</b>	Pharmaceuticals		
<b>Stage of development</b>	Basic research on mechanistic end point	Optimization method	In-house optimized Prevalidating
<b>Expected regulatory acceptance</b>	EC-ECVAM	OECD test guidelines	
<b>Expected interest from the market</b>	A producer technology company is already commercialising		

Project **PREDICTOMICS**

Contact: Jose Castell

<b>Test models included</b>	Toxicokinetic model	Genomic	
<b>Methods with commercial potential</b>	Assay for drug-induced liver cholestasis by flow cytometry	Multiparametric assay for druginduced steatosis	Nephrotoxicity
<b>Endpoints</b>	Choleostasis	Steatosis	Perfusion culture
<b>Expected field of application</b>	Pharmaceuticals: drug development, toxicity		
<b>Stage of development</b>	In-house optimized	Externally assessed prevalidated	
<b>Expected regulatory acceptance</b>			
<b>Expected interest from the market</b>	A producers would be convenient for marketing		

Project **COMICS**

Contact: Prof. Andrew Collins

<b>Test models included</b>	Enzymes-Receptors	Cell functions
<b>Methods with commercial potential</b>	DNA damage using the comet assay	DNA repair using the comet assay
<b>Endpoints</b>	Genotoxicity	
<b>Expected field of application</b>	Chemicals, Pharmaceutiucals	Food anf feed products
<b>Stage of development</b>	In-house optimized	
<b>Expected regulatory acceptance</b>	EC-ECVAM	
<b>Expected interest from the market</b>	End users are interested and supporting	

Project **EXERA**

Contact: Diego Di Lorenzo

<b>Test models included</b>	Toxicokinetic models	
<b>Methods with commercial potential</b>	New cell lines with non-tumoral from different tissues	Surrogate end-points
<b>Endpoints</b>	Toxikokinetics	Reprotoxicity
<b>Expected field of application</b>		
<b>Stage of development</b>	Basic research	
<b>Expected regulatory acceptance</b>	EC-ECVAM	
<b>Expected interest from the market</b>	A producer will be needed for marketing	

## Methods presented at Forum Event

At the Forum Event 12-14 May 2009 was a joint event of the EU-projects Forinvitox and Invitopharma and IVTIP and organised by Silverdal Science Park at Karolinska Institutet, Stockholm,

Sweden. The Following models with commercial potential were shown at the Forum.

<b>Organisation</b>	<b>Method</b>	
Nikon Instruments Europe BV,	Incubator with in-built robot, microscope, imaging system and analysis software.	
Biopredic International, Rennes, France	Use of HepaRG® cells for cytotoxicity and genotoxicity tests	
Biopredic International, Rennes, France	Use of HepaRG cells for enzyme induction, hepatic drug transport function, hepatotoxic effects	
Epithelix Sàrl, Geneva, Switzerland	Studying of human respiratory diseases and testing for effects on human respiratory tracts	
EggCentris, Brussels, Belgium	Battery of follicle bioassay (FBA), in vitro fertilization procedure (IVF) and the mouse embryo assay (MEA)	
EggCentris, Brussels, Belgium	Sperm Motility Assay (SMA) for detection of sperm toxicity Murine Leydig Tumor cells (MLTC-1) and primary rat/mouse Sertoli cells for testis specific toxicity	
EggCentris, Brussels, Belgium	Mouse Embryo Peri-Implantation Assay (MEPA) using mouse zygotes cultured to day 8 of development to study effects on early embryo development. The Embryonic Stem Cell Test (EST) study post-implantation embryo toxicity	
Institute for In Vitro Sciences, Gaithersburg, USA	Reconstructed Skin Micronucleus (RSMN) assay	

	<b>Contact</b>	<b>Title of abstract</b>
	Sundeeep Bhandari bhandari@nikonbv.nl	Nikon BioStation CT - an "all-in-one" toxicity testing system solution
	Christophe Chesné christophe.chesne@biopredic.com	HepaRG® human hepatocytes for toxicology studies
	Christophe Chesné christophe.chesne@biopredic.com	HepaRG® cell line: a new tool for drug metabolism and chemical -induced hepatotoxicity investigation
	Samuel Constant samual.constant@epithelix.com	The use of an in vitro cell model of the Human Airway Epithelium (MucilAir™) in preclinical development
	Rita Cortvrindt rita.cortvrindt@eggcentris.com	Battery of in vitro bioassays for the assessment of female fertility
	Rita Cortvrindt rita.cortvrindt@eggcentris.com	Battery of in vitro bioassays for the assessment of male fertility
	Rita Cortvrindt rita.cortvrindt@eggcentris.com	In vitro bioassays for the assessment of pre-and post-implantation embryo toxicity
	Rodger D. Curren rcurren@iivs.org	Development of a New In Vitro Genetic Toxicology Model Using Reconstructed Human Skin

<b>Organisation</b>	<b>Method</b>
Karolinska Institutet, Sweden	Use of Small Model Organisms (SMOs) as biosensors and Xenopus and Zebrafish embryos as models to study effects of endocrine disruptors and/or medical drugs.
Department of Neurochemistry, Stockholm University, Stockholm, Sweden	TRPV1-expressing SH-SY5Y cells
BioMed-zet-Life Science, Linz, Austria	Expression of CYPs in HepaRG cells, cultured using a porous plasma activated scaffold
Institut für Umweltmedizinische Forschung, Duesseldorf, Germany	Neurospheres from human and mouse fetal brains as a screening system for developmental neurotoxicity
British American Tobacco, Southampton, UK	Exposure of human lung primary epithelial cells to whole cigarette smoke
SymCel, Stockholm, Sweden	Energy turnover of isolated cells, tissues or organism assessed by monitoring their heat production by calorimetry
Cellial Technologies, Lens Cedex, France	Differentiated endothelial cell monolayer
F.C.V. Centro Investigacion Principe Felipe, Valencia, Spain	Flow cytometry and High-Content Analysis on 96-well miniaturized assays with 3 cell lines
CARDAM, (VITO NV), Mol, Belgium	Gene-expression (microarray, RT-PCR) in dendritic cells
TNO Quality of Life, Zeist, The Netherlands	A human skin model applicable for genotoxicity tests
Joint Research Centre, Institute for Health and Consumer Protection, Italy	Cultured networks of neurons (in vitro/ex vivo) for neurotoxicity evaluation

<b>Contact</b>	<b>Title of abstract</b>
Manuela Dahinden-Hase manuela.hase@ki.se	SME RECEPTOR - an industry-academia partnership for in vitro toxicology testing
Anna Forsby annaf@neurochem.su.se	The in vitro nociception assay for estimation of mild eye irritation
Jürgen Frank j.frank@zet.or.at	Development of an in vitro 3D-liver test system for the investigation of drug metabolism and long term drug hepatotoxicity
Ellen Fritsche ellen.fritsche@uni-duesseldorf.de	Neurospheres for developmental neurotoxicity (DNT) testing
Marianna D Gaca marianna_gaca@bat.com	A model of in vitro exposure to aerosols at the air-liquid interface
Dan Hallén dan.hallen@symcel.se	SymCel label-free and real-time data technology
Dorothee Hallier-Vanuxeem dvanuxeem@hotmail.com	Ready-to-use in vitro Blood-Brain Barrier model
Guadalupe Herrera compras@cipf.es	Multiparametric cytotoxic panel for in vitro cytotoxicity screening (MultiCytox-H®)
Jef Hooyberghs Jef.Hooyberghs@vito.be	VITOSENS™: a cell-based alternative to identify skin sensitizers by gene expression
Cyrille Krul Cyrille.Krul@tno.nl	The use of ex vivo human skin tissue cultures for safety testing
Antonio Novellino Novellino@jrc.itj	MEA based high-throughput electrophysiology for in vitro toxicology testing

<b>Organisation</b>	<b>Method</b>
F.C.V. Centro Investigacion Principe Felipe, Valencia, Spain	Combination of environment-independent, hermetic container to maintain longterm cell cultures and toxicity measurements by light or fluorescence microscopy
University of Vermont, Burlington VT, USA	Microscopic imaging by use of fluorescent reporter gene enabling specific organ/tissue development and metabolic pathways
Solidus, Biosciences, Troy, USA	DataChip analysis for evaluation of systemic and acute toxicity
PRIMACYT Cell culture Technology, Schwerin, Germany	Long-term culture systems for hepatocytes for repetitive analyses of metabolic and toxic effects of drugs
CARDAM, (VITO NV), Mol, Belgium	Tail coiling and swimming behavior as identification of developmental neurotoxicity
CIBERESP, Barcelona, Spain and Stockholm University, Stockholm, Sweden	GABAA receptor activity and Cell Membrane Potential in primary neuronal cultures and the human neuronal cell line SH-SY5Y.
Vrije Universiteit Brussel, Belgium	Liver-based in vitro models for screening for liver toxicity
BioThema, Stockholm, Sweden	Measurements of intracellular ATP levels used for cytotoxicity testing
Cellasys, Munich, Germany	Monitors parameters directly at living cells
ADVANCELL, Valencia, Spain	Reagent ISOCYP-TOX for in vitro screening of CYP450 biotransformation-mediated toxicity

<b>Contact</b>	<b>Title of abstract</b>
José-Enrique O'Connor compras@cipf.es	The use of PetakaG2™ hermetic cell culture containers and cytomics for testing of sustained in vitro toxicity (MultiCyttox-LT®)
Matthew D. Rand Matthew.Rand@uvm.edu	A Drosophila embryo platform for high-throughput testing of suspected toxins and bioactive compounds
David Rozzell rozzell@solidusbio.com	Miniaturized three-dimensional cell culture and metabolic enzyme arrays for high-throughput toxicity assays
Dieter Runge Dieter.Runge@primacyt.de	HEPAC: Serum-free, standardized and validated (re-usable) primary human hepatocytes for the analysis of xenobiotics
Ingrid Selderslaghs Ingrid.Selderslaghs@vito.be	The zebrafish embryo as an alternative model to screen for the developmental neurotoxic potential of chemicals and drugs
Cristina Suñol csenqi@iibb.csic.es	Combination of cell based assays determining GABA <sub>A</sub> receptor activity and Cell Membrane Potential for in vitro testing of neurotoxicity
Tamara Vanhaecke Tamara. Vanhaecke@vub.ac.be	Epigenetic modifiers as key tools to stabilise primary hepatocytes cultures and to produce progenitor-derived hepatocytes
Ida Westerdahl Ida.Westerdahl@biothema.com	ATP measurements in cell proliferation and cytotoxicity studies
Joachim Wiest wiest@cellasys.com	Label-free cell based assays as alternative testing method for toxicity
Maya R. Vilá toni@advancell.net	ISOCYP-TOX: A new ready-to-use concept for in vitro evaluation of biotransformation-mediated toxicity

# Manufacturers Offer

Expertrådet made an inventory of companies in Europe that are or could become involved in the production of alternative toxicity tests.

Company	Area of interest
<p>Advanced in vitro cell technologies, S.L. www.advancell.net</p>	<p>Biotech Company. Leader in Nanomedicin. Services and Reagents Unit: develops and markets in vitro models with a regulatory and/or predictive value. R&amp;D: develops new in vitro models and kits. Ready to use reagent line (www.cacoready.com)</p>
<p>Advanced CellSystems GmbH www.advancedcellsystems.com</p>	<p>Subsidiary of the Cell System®. Specialised in research, development of 3-D cell culture models In vitro assay development Toxicological in vitro studies Epidermal skin test (EST-1000) Advanced skin test (AST-2000)</p>
<p>BioDetectionSystems BV www.bds.nl</p>	<p>Bioassays for persistent organic pollutants (POPs) CALUX® systems for dioxins, estrogens, androgens hormones etc.</p>
<p>Biopredic International www.biopredic.com</p>	<p>Cells, tissues, fluids for drug assays PREDISAFE®, a cell kit for predicting eye irritancy of cosmetic raw materials and formulations</p>

<b>Company</b>	<b>Area of interest</b>
Biosafe-Special Laboratory Services Ltd. <a href="http://www.biosafeservices.biz">www.biosafeservices.biz</a>	Toxicity testing of chemically complex food contact materials. Cytotoxicity assay for acute and sublethal effects in combination with tests for genotoxicity
BioThema AB <a href="http://www.biothema.com">www.biothema.com</a>	Luminescent assays. Develops and produces reagents and kits for ATP analyses and reporter gene assays.
Biovator AB <a href="http://www.biovator.se">www.biovator.se</a>	Develops innovative in vitro tests for allergic sensitization. Cytokine Profile Assay (CPA) Gene Activation Profile Assay (GAPA)
BSL Bioservice Scientific Laboratories GmbH <a href="http://www.bslbioservice.de">www.bslbioservice.de</a>	CRO and consulting service. Biological trials of drugs, medical devices, cosmetics, biotechnological substances and chemicals. Toxicological studies in vitro Development of cellbased test systems

Company	Area of interest
CellSystems® Biotechnologie Vertrieb GmbH <a href="http://www.cellsystems.de">www.cellsystems.de</a>	Development of 3-dimensional cell culture models Skin models AST-2000, EST-1000 Blood-brain-barrier model, respiratory model angiogenesis model
EggCentris NV <a href="http://www.eggcentris.com">www.eggcentris.com</a>	Advice and technologies to screen compounds for their influence on fertility and embryonic development.
Fraunhofer Institut Toxikologie und Experimentelle Medizin (Fh-ITEM) <a href="http://www.item.fraunhofer.de">www.item.fraunhofer.de</a>	Contract research. Studies and risk assessment. Toxicological studies. Uses in vitro methods if possible as an alternative to animal experiments
Fundación GAIKER <a href="http://www.gaiker.es">www.gaiker.es</a>	Technological centre - research and providing innovation and technology services to companies.
Gentronix Ltd. <a href="http://www.gentronix.co.uk">www.gentronix.co.uk</a>	Innovative biotechnology. Develops technology for genotoxicity testing. Green Screen® products (cell based assays)
MicroBioTests Inc. <a href="http://www.microbiotests.be">www.microbiotests.be</a>	Bioassays for toxicity screening of surface and ground- waters, effluents, sediments, contaminated soils, solid
Noray Bioinformatics, S.L.U. <a href="http://www.noraybio.com">www.noraybio.com</a>	IT support to biosciences sector (softwares)

Company	Area of interest
NV reMYND <a href="http://www.remynd.be">www.remynd.be</a>	Biotech spin-off of the University of Leuven, Belgium Field of Alzheimer's and Parkinson's disease. Contract research and drug discovery
StratiCELL <a href="http://www.straticell.com">www.straticell.com</a>	In vitro models for tissues, toxicity efficacy, ageing research. Screening technologies.
Tebu-bio <a href="http://www.tebu-bio.com">www.tebu-bio.com</a>	Reagents, lab services and softwares for life sciences. Gene expression, cell biology, signal transduction, drug discovery, ADME-tox.
VITROCELL Systems GmbH <a href="http://www.vitrocell.com">www.vitrocell.com</a>	Solutions for cultivation and exposure for in vitro studies of gases or complex mixtures.
Xenometrix AG <a href="http://www.xenometrix.ch">www.xenometrix.ch</a>	Production, distribution and services of mutagenicity test systems (Ames II Mutagenicity Assay), and cytotoxicity screening test kits (IN CYTOTOX). Use in molecular pharmacology, early stages of drug discovery, screening of chemical and agro chemical compounds, environmental projects

# Producer Capacity

The inventory made by Expertrådet also comprised to learn about the manufacturers strengths and weaknesses to meet future demands. The inventory showed that the largest group of customers is the pharmaceutical industry with 28% of all answers. Also the chemical and cosmetic industries are large categories of customers for the in vitro/in silico companies, with 16% and 12% of the answers respectively. Other customer groups are CROs, research groups, biotech companies, environmental testing, food industry, authorities, paper and board industry, plastic industry and tobacco industry.

Companies in Europe involved in manufacturing in vitro and in silico methods cover a broad spectrum of endpoints and categories of customers. However, more than 50% of the responding companies assert that they have capacity to test more than they do today. The basic requirements needed for an invention to be further developed into a commercial product is that it is reproducible, regulatory accepted, practical, low cost, and has screening capacity.

The opinion of the manufacturers are that the following tests and endpoints are urgently needed to meet future testing demands.

- Reproductive and developmental toxicity
- Skin sensitisation
- Genotoxicity
- Phototoxicity
- Skin corrosion
- Skin irritation
- Allergenicity tests
- Blood-Brain Barrier permeability tests
- Carcinogenicity assays
- Chronic toxicity
- Combined cytotoxicity tests
- Human toxicity
- Integrated tests combining genomic/proteomic/metabolomic data
- Immunotoxicity
- Inhalation toxicity
- Respiratory toxicity
- Mutagenicity tests
- Neurotoxicity tests
- Safety tests
- Teratogenic toxicity

# User Needs

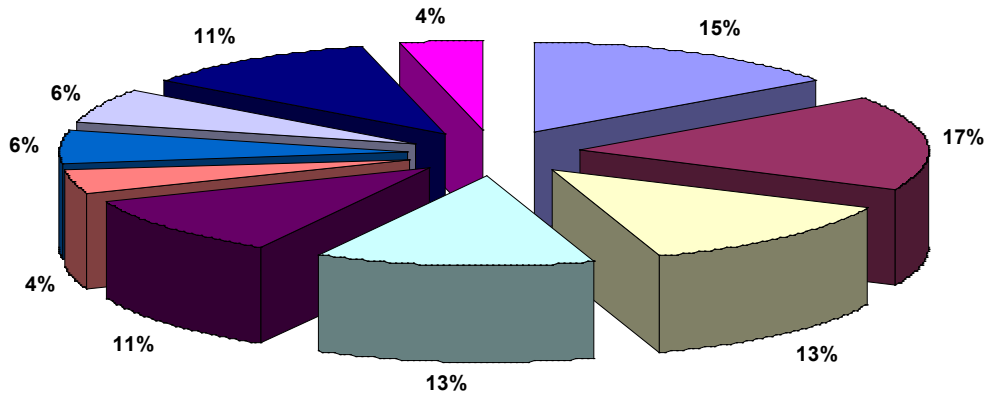
IVTIP was in charge of collecting the needs of the end-users. For that, a questionnaire was prepared and sent to all IVTIP members and companies belonging to the IVTIP network. A series of new approaches were proposed that could be summarised in three aspects which should be improved: (A) Need to increase com-

plexity by the use of multimetric end-points into the simple cellular models; (B) Incorporate complex models in the testing battery; and (C) Implement more “human” testing models, more related to the final target.

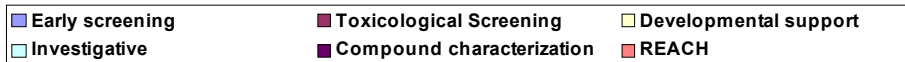
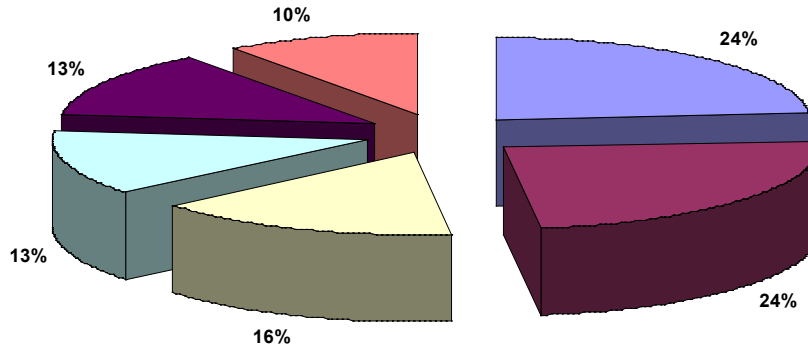
## Future needs for in vitro testing

<b>Technical approaches</b>	<b>%</b>	<b>New technologies</b>	<b>%</b>
Simple studies (single end-point)	27	Genomics	39
Relatively complex studies (single end-point with different time-points or several end-points with a single time-point)	46	Proteomics Cellomics Metabolomics	24 15 15
Complex studies (several end-points with different time-points)	27	Other	7

The models used by the final users today is reported in the following figure



The main objectives for using in vitro methods/models are reported in figure below.



# Presentation of the participating organisations

## The Silverdal Science Park

www.silverdal.se

Silverdal Science Park is a non-profit association with the following objectives:

- To increase the number of usable and validated alternative methods according to the 3Rs.
- To increase the mutual knowledge of alternative tests between end users, manufacturers, regulatory agencies and researchers.
- To inform about and influence on the general regulation and policy regarding alternative tests.
- To coordinate and initiate research and development of new alternative methods.

The intention of the association is to create a network and meeting forum for stakeholders as researchers, end-users, manufacturers and regulatory agencies to strengthen the opportunities for the use and development of new alternative methods. The association is financed mainly by the companies Skanska and JM.

Today the association has 20 member organisations from companies (e.g. AstraZeneca, Gambro and Cellartis), authorities (e.g. The Swedish Chemicals Agency) and scientists that develop animal free methods that can be used in consumer product safety testing.

Among the activities within the Silverdal Science Park the following seminars have been arranged:

- Presentation of EC funded projects with the aim to develop new in vitro methods for toxicity testing
- Possibilities to finance development of toxicological in vitro methods
- How is the legal authorities approach in vitro toxicity methods
- Do today's in vitro tests meet the future demand of cosmetic testing?
- Bottlenecks in commercialisation and use of in vitro tests

The Silverdal Science Park is a partner of the FP6 projects Invitopharma and Forinvitox.

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## REMA

[www.remanet.net](http://www.remanet.net)

REMA, the Spanish Network for Alternative Methods is a non-profit organisation involving the 4 parties (Academia, Government, Industry and Animal Welfare Organisations). REMA acts principally as a channel to coordinate different initiatives concerning the 3R's Concept in Spain. It promotes the organisation of specific scientific meeting, disseminate and promote the development of alternative methods among the scientific community and collaborate with Government for advise in new regulation of animal experimentation and registration of drugs, cosmetic and chemical products. REMA is registered with the name of "Red Española para el Desarrollo de Métodos Alternativos a la Experimentación Animal-REMA" in the Ministry of Internal Affair as a non profit organization with the national number 169901 of the Section 1 and registered in the Ministry of Economy as juridical entity with Identification Code (NIF): G-83486175. His official postal address is "C/Serrano 115, Madrid"

Connections with International Organizations. The REMA is a foundational member of ECOPA, the European Consensus Platform on Alternatives, and its Vice president, Dr José Castell is member of the REMA Coordination Committee. The REMA structure and procedure for constitution and organization has been used as model to develop other National Platforms in other countries. REMA maintain fluid relationship with the European Centre for the Validation of Alternative Methods (ECVAM) and Dr. José Castell is serving as an expert in different Task forces in ECVAM. Dr Argelia Castaño is member of Scientific Advisory Committee, and a scientific officer of ECVAM, Dr Pilar Prieto is member of the REMA Coordination Committee.

Participations in European projects and initiatives. REMA is a National Platform member of ECOPA and an active member of the CONAM project, funded by EC under 6FP.

## SET

[www.stiftung-set.de](http://www.stiftung-set.de)

The central aim of the SET Foundation is to reduce and replace animal experimentation. The Foundation consists of representatives from industry, animal welfare, science and government. Their role is the transparent, interdisciplinary allocation of funds to eligible projects researching and implementing methods to replace and complement experiments on animals.

Following an initiative by the German Federal Ministry of Food, Agriculture and Forestry, the SET Foundation was established on 21 March 1986. The abbreviation SET stands for Stiftung zur Förderung von Ersatz- und Ergänzungsmethoden zur Einschränkung von Tierversuchen, - Foundation for the promotion of alternate and complementary methods to reduce animal experiments. Its approach was revolutionary, bringing together representatives from different groups for the common goal to reduce or avoid animal experiments:

- Bundesverband Tierschutz e.V.
- Deutscher Tierschutzbund e.V.
- Industrieverband Agrar
- Industrieverband Körperpflege- und Waschmittel
- Verband der chemischen Industrie
- Verband der forschenden Arzneimittelhersteller

Initial funding came from the participating industrial groups. The funds for eligible research projects come from donations and from interest on the Foundation's capital. The Foundation can look back on more than 50 successfully completed projects. The SET Foundation helps industry, animal welfare organisations, research bodies and government to co-ordinate their efforts and work together towards their common goal.

The Foundation concentrates its support on three main areas:

- Scientific communication regarding alternative and complementary methods already available and in development.
- Developing and implementing new 3Rs methods, particularly in areas where stressful animal experimentation is still necessary or where scientific routine impedes 3Rs methods.
- Measures with the potential to extend the use of alternative and complementary methods in scientific research, such as implementing 3Rs methods in student training and further education.

In order to maximise their effect in the scientific and industrial environments, the SET Foundation works to establish 3Rs methods with as many potential users as possible. Funding therefore extends beyond research projects, to publications, symposiums, training courses and workshops.

## IVTIP

[www.ivtip.org](http://www.ivtip.org)

The In Vitro Testing Industrial Platform (IVTIP) is an association composed of European companies with an active interest in in vitro testing, not only to be used in regulatory safety testing but also for compound discovery and product development. Member companies represent the pharmaceutical, chemical and cosmetic sector, as well as independent contract research organizations. The members of IVTIP are all active in supporting and applying the principle of the 3Rs (Replacement, Reduction and Refinement of animal testing), and in promoting the adoption of the fourth R (Responsibility in research promoted by industry). IVTIP is a science-driven organization existing for and by the members.

The representatives in IVTIP are scientists who are active in the area of in vitro and/or in silico methods. IVTIP provides advice to European Union (EU) Institutions about industrial activities and needs for research, development and application of alternatives to animal testing. Vice versa, they inform industry about upcoming EU activities and new regulations involving in vitro testing. IVTIP is an active group of scientists, who discuss new opportunities, inform each other about promising developments and participate in EU projects. The liaison with academic groups is very important to stimulate the applicability of techniques and methods for industrial use, thereby ensuring effective dissemination through transfer of

both technology and knowledge

### Board Members:

Erwin Roggen	Novozymes A/S, Denmark
Cyrille Krul	TNO Quality of Life, The Netherlands
Bart De Wever	Henkel, Germany
Klaus Schröder	Henkel, Germany
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Myriam Fabre	Advancell, Spain
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Marianna D Gaça	British American Tobacco, UK

### Expertrådet

Expertrådet - a consultant company with expert knowledge in toxicology. The three major fields of activities of Expertrådet are:

- investigations and information in environmental and health issues
- coordination of extensive research projects
- advice in matters concerning commercialisation of research results







