

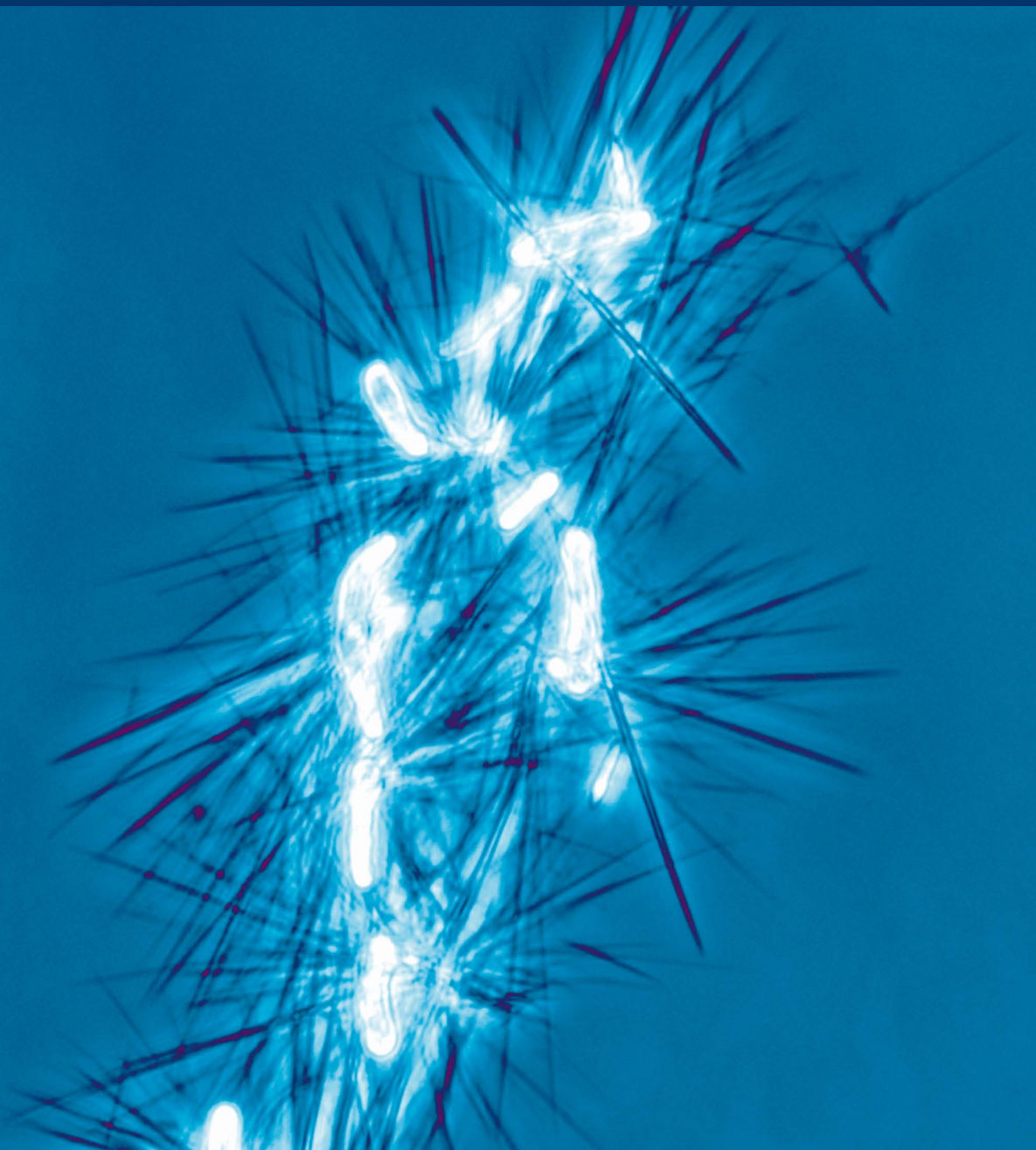


**Fraunhofer**

**LIFE SCIENCES**

FRAUNHOFER GROUP FOR LIFE SCIENCES

# **REACH CONSULTANCY AND SUPPORT**



# REACH





# THE EUROPEAN UNION REGULATION ON CHEMICALS

REACH, the European Union regulation for the **R**egistration, **E**valuation and **A**uthorization of **C**hemicals, requires manufacturers and importers of chemical substances to register these materials with the central European Chemicals Agency (ECHA) before bringing them to the market in quantities of one tonne or more per year.

Aiming to provide a high level of protection of human health and the environment, the safety of production and use of registered chemicals produced or imported in quantities of more than ten tonnes per year has to be demonstrated by the registrant in a Chemical Safety Report.

The transfer of information up and down the supply chain is a key feature of REACH. Depending on the manufactured or imported quantity of a chemical, potential risks and measures to control these have to be communicated down the supply chain. Likewise, information flow in the reverse direction – from users to manufacturers – about, for example, their specific use and exposure conditions, is also an indispensable aspect of communication in the supply chain.

Without registration with the ECHA, marketing and commercialization of a chemical in the EU are strictly prohibited.

## We answer your questions

The current REACH regulation is complex and there are many questions that need answering in advance of and also after registration.

### *Prior to registration:*

- Which of your materials are affected by REACH?
- Why is it advisable to start planning your registration according to the 2018 deadline now?
- What quantities do you produce and what are the associated requirements regarding information?
- What relevant information is already available in your company and what additional information is available in the public domain or other regulatory systems?
- Has your chemical already been registered by another company and if so, how can you benefit from this?
- Are there any other companies planning registration and how can you cooperate and share costs with them?
- Are any obligatory data missing and what strategies and measures can be used to get around this?
- What experimental studies are unavoidable?
- Do your safety data sheets comply with the current requirements?
- How to classify and label your chemical according to CLP/ GHS? Are there any other C&L systems available for your substance and what relevance do they have?

### *After registration:*

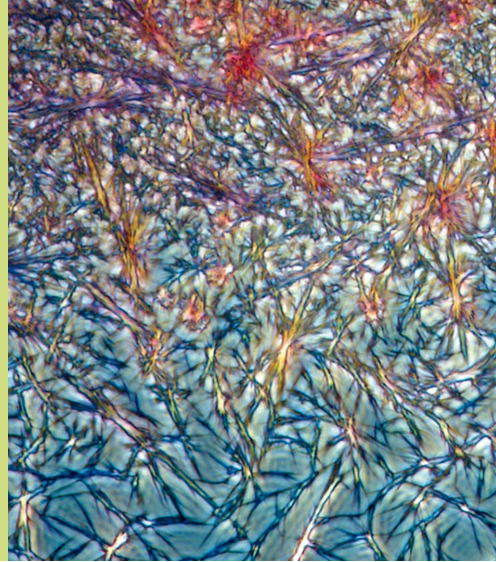
- Your registration dossier has been checked by the authorities. What is the best way to address ECHA's draft decisions?
- ECHA defines registrations under REACH as "living dossiers" that need continual attention. What are your obligations after registration and how can you best handle them effectively with minimum effort?

These are just a few representative questions. Further questions that will come up during your specific project will be answered by us.



# INTELLIGENT TESTING STRATEGIES





## MINIMIZING EXPERIMENTAL STUDIES

The Fraunhofer Group for Life Sciences has many years of experience in chemical risk assessment. The cornerstone of our expertise is an interdisciplinary team of scientists who have successfully registered a variety of important chemicals under various regulatory regimes, often on behalf of international consortia. Collaborative work within the Group and with external partners allows us to test chemical compounds used in a wide range of applications, gather and evaluate the required data, and prepare all relevant documents. By adopting intelligent testing strategies and using all available data sources as well as QSAR (quantitative structure-activity relationships), we proceed step by step towards the objective of minimizing experimental studies and financial burden.

In collaboration with you we determine to what extent chemical substances of your portfolio fall under REACH. Thereafter, the documents available at the company are screened and checked to determine whether the required physico-chemical data, toxicological information, and details of ecotoxicity and environmental fate are available. On the basis of this information it can be determined whether there are any data gaps.

One main REACH principle is that for any one substance, a single set of information on its intrinsic properties is produced which is shared by all those companies that manufacture or supply this substance. We will thus check first whether your substances have already been registered or registrations are planned by other companies. Having considerable experience in joint registration and data sharing on behalf of and within consortia, we figure out the best and most cost-saving way for you to benefit from an optional cooperation.

The utilization and linking of existing knowledge are efficient measures for filling data gaps. The first step here is to use scientific literature, assessment dossiers, and other publicly available

sources. Thereafter, data are generated via in-silico methods such as the evaluation of structure-activity relationships, read-across (namely utilization of data for substances with similar chemical structures), and chemical categories and analogy concepts. We also investigate whether waiving, namely the justified non-performance of some tests, can apply here.

In standard exposure scenarios the potential exposure for man and the environment is reflected by modeling the real-life handling and applications of chemical substances or products. A scenario also gives information about how a substance is released and the routes via which it can be taken up into the body: via the skin (dermal), the mouth (oral), or the respiratory tract (inhalation). Environmental scenarios give information about the behavior and fate of the chemical substance in the environment.

If there are still data gaps at this stage, we will jointly clarify what tests must be undertaken. Our scientists are able to carry out the full spectrum of standard tests. We will develop intelligent testing strategies for you in order to acquire the data required by REACH. Where possible, in-vitro methods will be used as an alternative to animal tests.

Taking into account all the available information and the test results we will draw up an evaluation and prepare all documents required for the registration.

Many years of experience dealing with relevant authorities, active participation in panels accompanying legislation, and collaboration with national and international scientific organizations guarantee that your registration will be based on the current state of knowledge.



# TAILOR-MADE CONSULTANCY





## SUPPORT THROUGH TO SUCCESSFUL REGISTRATION

The REACH regulation is complex and the registration procedure is strict. You should not leave anything to chance, because the success of your business depends on successful registration of your chemicals. We organize the registration procedure for you in an understandable and transparent way. You can rely on our practical knowledge and interdisciplinary expertise. The Fraunhofer Group for Life Sciences can advise you from the very beginning right through to successful project completion, through all phases of the REACH regulation.

Following a structured plan of the process, we will proceed step by step towards the goal. We know what sub-tasks must be completed, when they must be completed and how, and you maintain a view of the steps that still lie ahead. This is of great benefit for reliable planning and transparency and particularly so for projects involving hazardous chemicals or large production quantities.



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### Consecutive steps

Collection of available data by the manufacturer/importer

Check for cost sharing options (e.g. joint registration)

Literature survey, data collection

Evaluation of the data, IUCLID update

Data gap analysis

Proposed strategy for data generation

Discussion of the strategy

Close collaboration and communication in the SIEF

Generation of non-experimental data (SAR, read-across, chemical categories, waiving)

Determination of the exposure (scenarios, data from the product chain)

Preparation of the registration dossier in accordance with Article 10:

Technical dossier

Chemical safety report  
> 10 tonnes per year  
> 1 tonne per year for CMR, PBT, vPvB

Communication with the authorities and ECHA, discussion of the dossier and proposed tests

Execution of experimental studies, if necessary

Communication of the study results to the authorities

Successful registration

Support after registration, e.g. commenting of the draft decision regarding any proposed experimental testing; discussion with ECHA, dossier update



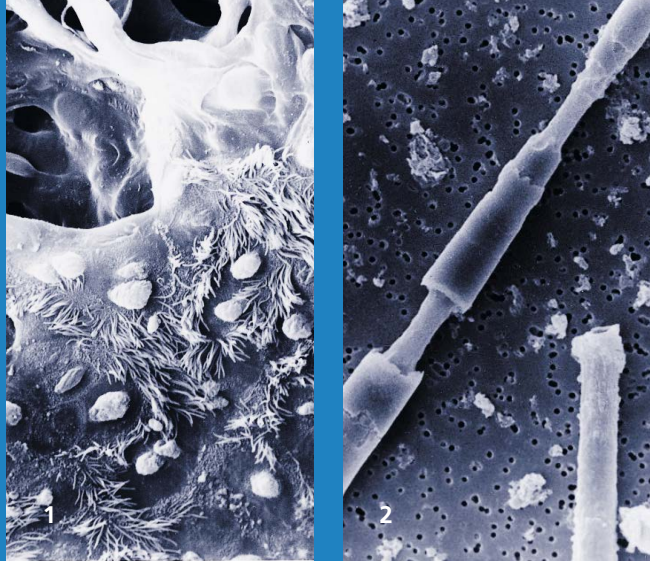
# EFFECTS AND BEHAVIOR OF CHEMICAL SUBSTANCES





1 Bronchiole with cilia and secreting cells.

2 Fibers.



## CLARIFICATION OF (ECO-)TOXICOLOGICAL ISSUES

The REACH regulation guarantees a high level of protection for people and the environment. Information is required about the behavior and fate of a substance in the environment (mobility) and about its toxicity to people and organisms. We use data that is already available and develop substance-specific strategies to generate valid data for endpoints for which experimental data are not available (QSAR, read-across, chemical categories, waiving). You benefit from our expertise in clarifying toxicological issues: acute, subacute, and chronic effects can be tested for the relevant uptake pathways.

### Quantitative structure-activity relationships (QSAR)

QSAR are methods which allow determination of the primarily structure-dependent properties of a substance and can help fill existing data gaps. Results of applying validated QSAR models may indicate the presence or absence of certain dangerous properties, for example biodegradability, adsorption potential, lipophilicity, or bioaccumulation potential. With the help of extensive databases on effects and toxicological studies, an expert system for read-across and chemical categories has been established.



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### Exposure assessment

Exposure assessment is important in order to estimate the concentrations of substances, for example at the workplace and in the environment (water, soil, or sediment). These concentrations, together with the relevant data on the effects, are the basis of the risk assessment. Calculating the concentrations is based on a combination of exposure categories regarding the primary entry of the substance into the environment during its production or use and the subsequent distribution based on its chemical properties.

With your help we set up the required use and exposure categories for manufacturers, importers, and downstream users. If required, we develop customized exposure scenarios to predict the behavior of your substances in the environment and to assess workplace and consumer exposure. Whenever refinements in the calculation of environmental exposure become necessary, we perform all the higher-level calculations accepted by the authorities, using the different EU FOCUS models.



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1 *Myriophyllum aquaticum*.

2 Pair of minnow.



## EXPERIMENTAL STUDIES

If experimental studies are required in order to fill data gaps, these are carried out in accordance with test procedures which are accepted by the authorities.

The spectrum of methods ranges from standard tests in accordance with international guidelines (e.g. OECD or EU) to complex studies for solving very specific problems. All tests required by REACH can be carried out by member institutes of the Fraunhofer Group for Life Sciences, where necessary in collaboration with reliable external partners, under GLP conditions. Many years of experience in dealing with German national authorities (Federal Institute for Occupational Safety and Health, Federal Institute for Risk Assessment, Federal Environment Agency) and international authorities facilitate the drawing up and discussion of dossiers. We have particular expertise in the following fields:

### Toxicology

- Inhalation toxicology (acute, subchronic, and chronic tests; aerosols of particles and fibers, including nano-structured particles; droplet aerosols; complex mixtures)
- Genetic toxicology (mouse lymphoma assay, micronucleus test, chromosome aberration test, comet assay in vitro)
- Reproductive toxicology (fertility, teratogenicity, perinatal and postnatal development)
- Associated studies (clinical chemistry, histopathology, analytics, toxicokinetics)



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### Ecotoxicology

- Long-term and short-term ecotoxicity studies required for substances in accordance with Annexes VII to X and for categorizing and classifying a substance. These tests are also performed to test problematic substances, for example, using water-accommodated fractions (WAF).
- Studies to refine the PNEC in order to allow a more realistic estimate of the risk quotient (e.g. individual species tests under realistic exposure conditions, species sensitivity distributions, microcosm and mesocosm studies in cooperation with gaiac and Mesocosm GmbH)



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### Behavior in the environment

- Substance-specific data (e.g. investigation of transformation and solution behavior)
- Mobility and degradability in soil or sediment
- Bioaccumulation potential, determination of the PBT properties
- Bioavailability tests in different media



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# ALTERNATIVE TEST SYSTEMS





# CELL-BASED AND TISSUE-BASED TEST SYSTEMS

One objective of REACH is for base toxicological information to be acquired by using cell-based in-vitro test methods as alternatives to animal experiments. The Fraunhofer Group for Life Sciences fully supports this objective.

## **Cell-based arrays as in-vitro test systems for multi-compound exposure (MCE)**

Compared to artificial systems, cell-based systems have the advantage that the complex biochemistry of the cells and thus their natural ability to synthesize and also their ability to react to substances can be utilized. This enables rapid identification of dose-response relationships and derivation of certain limit and threshold values.

By using state-of-the-art technologies such as micro-arrays, it becomes possible to not only study the effects of single substances on living cells in a reproducible and highly accurate manner, but also to test a variety of substances at the same time. Such cell arrays thus enable fast and inexpensive multi-compound exposures (MCEs). Not only do they allow testing of several different compounds at once, but also the use of different cell types to study the effects of the test substances on different systems simultaneously.

A next step will be the coupling of such MCE chips in cartridge systems, which could then be used as mobile lab units for on-site measurements, providing additional options and more flexibility in toxicity analyses.

A "cell chip" is created according to the following scheme:

- Surface modification of the substrate (glass, COC, PS etc.) or structuring
- Cell patterning by spotting or structured growth (using stencils or defined growth areas)
- Addition of the analyte
- Data acquisition by using array scanners or microscopy techniques

Cell-based arrays have the advantage that the toxicity of different chemicals or other potentially harmful substances can be tested in a space-resolved manner on a very small area or, depending on the aim of the analysis, that different cell types can be exposed at the same time to a particular test substance.

We offer:

- Substrate customization for a specific cell type, using a wide range of formats (glass chip – MTP)
- High-throughput measurement of different analytes (e.g. drug screening)
- Data acquisition and evaluation by means of array software (e.g. Array-Pro)



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1 Automated hanging-drop (HD) cell culture using special HD plates.

2 Cells cultured on modified alginate microcarriers. Top: phase contrast; bottom: fluorescence staining to evaluate cell viability.

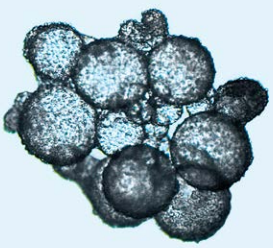
### Miniaturized high-throughput screening of human pluripotent stem cells

At present, cell-based screenings are an essential basis for all types of clinical developments and for authorization of new drugs and chemicals. Over the past decade, a substantial shift occurred towards physiologically more relevant, yet very complex and sensitive cell models, such as pluripotent stem cells. Because of their extraordinary potential to form any cell type of the human body, much hope is pinned on human pluripotent stem cells in the area of regenerative medicine. As model systems for cytotoxicity tests and drug development they have become virtually non-substitutable. The discovery of induced pluripotent stem cells (iPS), leading to Gurdon and Yamanaka being awarded the Nobel Prize in 2012, has improved both the availability and disease specificity of human pluripotent stem cells. At the same time, ethical concerns like those in the context of human embryonic stem cells (hESC) are unfounded here. iPS cells are generated by introducing specific genes into cells from the organism, for example from a skin biopsy, thereby artificially reprogramming these cells to the pluripotent state. As a result, the cells are again able to differentiate into cell types of all three germ layers. The pharmaceutical industry in particular has chosen this cell type as ultimate test system for the development of new active agents, given that these cells can be produced specifically for different disease patterns

with their particular characteristics and mutations. In the future, iPS cells furthermore will enable development of personalized treatment strategies, after preliminary testing of the most efficient and least stressful treatment in patient-specific cells.

For numerous toxicological risk assessments, three-dimensional cell aggregates or micro-tissues are used to test chemicals and drugs, as they alone allow a realistic assessment of efficacy. For the native function of cells, the three-dimensional environment plays a vital role and for stem cell differentiation in particular also the micro-environment (so-called cell niches). Many studies have shown that findings from monolayer cultures are not transferable to medical applications. A cell culture method that eliminates this drawback is the hanging-drop method, where a small volume of a defined cell suspension (approx. 20  $\mu$ l) is pipetted into the lid of a Petri dish. Once the lid has been turned upside down, the cells accumulate at the bottom of the drop and form a homogeneous cell aggregate in a defined micro-environment that is even suitable for cultivation of embryonic stem cells. Due to the complicated handling of the drops on the lid interior, however, automation is impossible. This problem can be solved with special perforated plates, allowing individual drops to be placed from above by pipetting robots. Change of medium, the adding of factors or even growth surface by micro-carriers are no longer any limitations. Using this method, we were able to preserve pluripotency of human iPS





cells during 10 days of automated hanging-drop cultures on microcarriers. Addition of growth surfaces allows control of the adhesion, enabling even sophisticated differentiation protocols: cardiac differentiation, for example, requires 3-day suspension culture to be followed by adherent cultivation. Each drop corresponds to a self-contained micro-bioreactor that can be accessed individually by pipetting robots and has optimal gas exchange, so that parallel and miniaturized high-throughput tests in a three-dimensional environment are feasible. (BioSpektrum 05.13, Springer-Verlag 2013, doi: 10.1007/s12268-013-0351-8)



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#### New technologies

Research and development work is our expertise and we therefore understand the limits of technology. But we are also aware of the wishes of our customers. Closing the gap between one's wishes and reality is what drives us to improve existing methods and procedures. By combining biological cells with technical microsystems, we provide access to novel assay technologies. These new technologies, for example, enable cell-based tests with increased sensitivity and reproducibility. Tests at the single-cell level and long-term studies of cell behavior at relevant concentrations of active substances are possible.



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1 Cytotoxicity testing in the laboratory.



## IN-VITRO TISSUE MODELS AS TEST SYSTEMS

We have developed three-dimensional in-vitro tissue models which can help reduce the number of animal tests required. Each model possesses many of the typical properties which are a feature of the relevant body organ. Your advantage as a customer: you receive near-reality bio-analyses of the toxicity of chemicals (cell toxicity, genotoxicity, embryo toxicity).

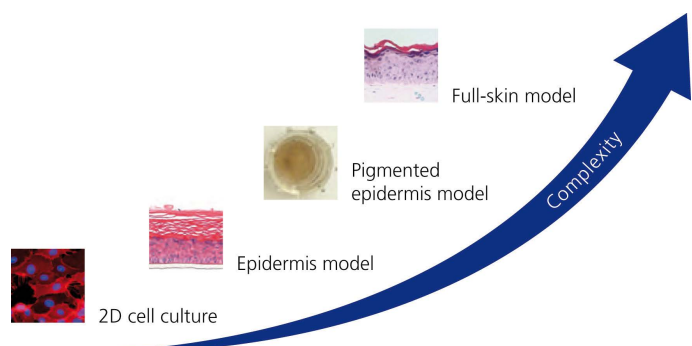
In addition to our 3D tissue models, we also use standardized 2D cell cultures. You can thus choose from several cell and tissue models: skin, bowel including a 3D colon carcinoma model, trachea, lung and 3D lung carcinoma, central nervous system, and stem cell differentiation (e.g. embryo toxicity test). Using the 2D and 3D tissue models as well as individual cells we are able to test many substances in a short space of time and hence acquire a wealth of information.

### Standardized in-vitro skin model

The artificial skin is the first standardized in-vitro test system that is configured like complex human tissue. Constructed from human cells, the patented three-dimensional skin equivalent (patent no. EP 1 290 145B1), like natural skin, has a dermis and epidermis layer. The epidermis layer contains the typical cornified envelope, making this skin model ideal for toxicity and corrosivity testing of chemicals. The standardized full-skin model can furthermore be customized for other specific investigations. We are thus able to provide our clients with a custom-made and informative test system.

### Skin test systems of different degrees of complexity – tailored to specific needs

The Fraunhofer IGB is accredited for cytotoxicity testing according to DIN ISO 10993-5. We use cell lines, but also the complex 3D full-skin model for this purpose. In addition, for the testing of novel substances we have a variety of other in-vitro skin test systems of different degrees of complexity, which we customize whenever needed to meet the specific requirements of each client. The irritant, corrosive, or phototoxic potential of a substance, for example, is determined in 3D epidermis models, taking into account the relevant OECD guidelines (irritation: TG 439, corrosion: TG 431, phototoxicity: TG 432 and INVITTOX Protocol No. 121). By integrating melanocytes into the skin models, we can also test substances promoting or inhibiting melanin synthesis in skin cells, such as skin tanners or skin bleachers. Quantification of melanin production allows us to verify in addition whether a substance has a protective effect against UV radiation.



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1 Automated production of tissue tests in the "skin factory".

### Factory-made skin

The chemicals policy REACH has substantially increased the need for in vitro produced tissue models. The demand can no longer be satisfied with the common manual production methods. For validation of artificial skin by the competent authority – the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) – a human epidermis model has been selected which by now can be produced in a fully automated process. This multi-stage process is performed entirely within a single modular production plant. Human epidermal cells (keratinocytes) are introduced into the plant, propagated (expanded), and seeded onto appropriate carrier membranes. After little less than three weeks of culture in an incubator, artificial epidermis can be harvested. This method allows reproducible large-volume production of high-quality epidermis models which morphologically do not differ from manually produced ones. After successful validation of the epidermis model, the "skin factory" is planned to produce about 5000 units per month for worldwide commercialization. The "skin factory" has been developed by four Fraunhofer institutes, coordinated by the Fraunhofer IGB, in a project funded by the Fraunhofer-Zukunftsstiftung (Fraunhofer Future Foundation).



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### Intestinal model – absorption studies at the intestinal barrier

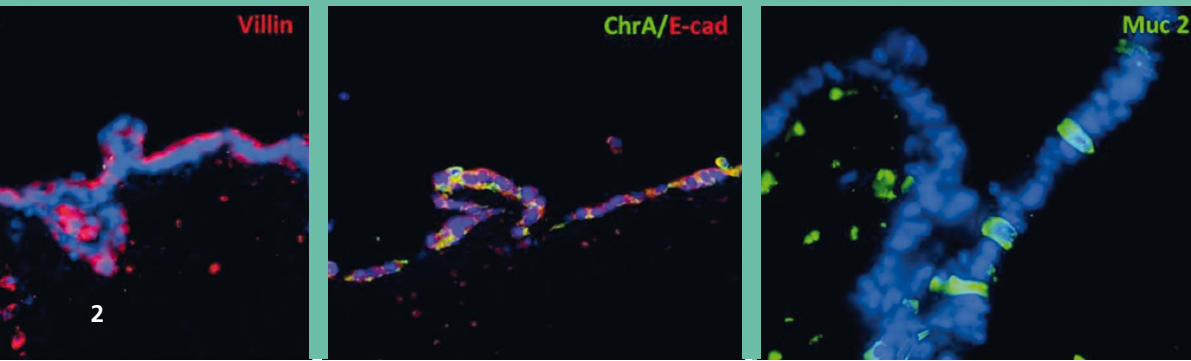
A decisive factor for the efficacy of an orally administered pharmaceutical is the question how well the substance is absorbed by the intestinal epithelium, before it gets to its actual site of action via the bloodstream. To study the absorption, toxicity, and bioavailability of orally administered active agents, the Fraunhofer IGB uses a standardized two-dimensional in-vitro model. It is based on CaCo-2 cells (colon carcinoma cells) cultured on an artificial PET insert membrane. The classification of substance transport by means of the CaCo-2 model is an accredited "own-label" method of the Fraunhofer IGB.



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2 Immunohistochemical characterization of an intestinal barrier model.

Bilder: Matthias Schweihin

### 3D colon carcinoma model

Our three-dimensional (3D) tumor model of colon carcinoma is based on a decellularized intestinal matrix combined with different tumor cell lines. To test the effects of targeted or cytostatic agents, we use selected cell lines, either individually or in combination. Since the 3D model usually has a lower cell division rate than 2D models, it yields a lower false-positive error rate in the testing of cytostatic agents. Besides the division rate and apoptosis of tumor cells, we can measure activation and inhibition of different signaling cascades upon treatment with a particular drug. Co-culture with cells from the tumor environment additionally provides the possibility to study active agents that influence the interaction of tumor cells with their surroundings. Such interaction is believed to play an important role in tumor cell invasion into adjacent tissue. Our tumor model thus allows the mechanisms of novel therapeutic strategies to be tested in a complex environment.



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### Intestinal models based on primary cells

In absorption studies and in the elucidation of novel mechanisms, the use of two-dimensional monolayer cultures and cell lines alone reaches its limits, because the composition of intestinal tissue cell types, the cell-cell contacts, and the formation of functional cell aggregates does not appropriately mimic the in-vivo situation. To enable investigation of more complex interactions in the future, we have developed tissue models that are based on primary cells. We meanwhile have at our disposal mouse and human intestinal barrier models and organoid tissue cultures. Both these systems include stem/progenitor cells and the various types of differentiated intestinal cells (i.e. enterocytes, goblet cells, enteroendocrine cells, and M cells). Besides characteristic molecular markers, the cells develop a polarized phenotype and form a physiological barrier that is further improved under dynamic flow conditions in a bioreactor. Primary intestinal models are another important contribution to pre-clinical screenings and could help reduce costs and animal experiments.



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1 Cornea.

### Cornea model

In cosmetic, drug, and toxicological research, non-animal models are needed that allow demonstration of responses to applied or administered substances and of therapeutic and protective effects. Ex-vivo cultivation of cornea from slaughtered pigs is a method which can help reduce in-vivo animal experiments. Substances are tested according to an adjusted OECD 405 protocol. The model enables detection of acute lesions caused by poisons, corrosive substances, and mechanical or physical action; but chronic alterations and whether or not tissue regeneration is possible can likewise be determined. The cornea model allows repeated exposure to substances, as common with eye drops and cosmetics, and investigation of the effects. To enable cultivation of the cornea close to the in-vivo situation, a special bioreactor has been developed. Superficial alterations and also those in the deeper cornea can be demonstrated with special methods (microscopy, optical coherence tomography, impedance, histology, fluorescence).



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### Trachea model

Man is the only natural host of the causative agent of pertussis (or whooping cough), *Bordetella pertussis*. This bacterium colonizes epithelial cells of the airway mucosa in the trachea and bronchi, causing a serious infectious disease in young children in particular. Elucidation of the mechanisms of disease and vaccine development is based mostly on animal experiments, however, most of the data generated in these experiments cannot be transferred to humans. Consequently, there is a great demand for appropriate tissue models of human airway mucosa. We have successfully established such a 3D test system, which closely mimics the natural airway tissue (Figs. 2 A-D).

Analyses by optical or electron microscopy have shown that the epithelial layer of our tissue model includes all relevant cell types present in vivo in tracheobronchial epithelium:

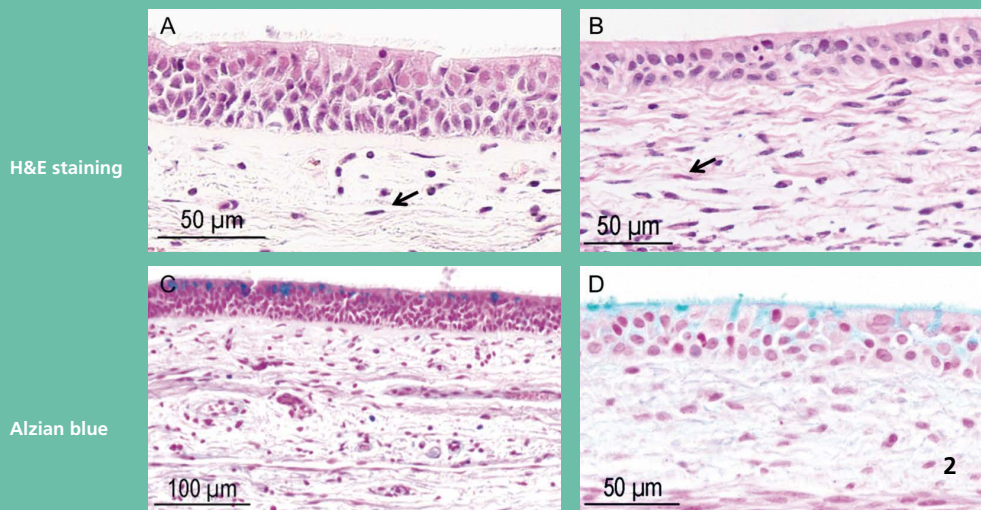
- Basal cells, which are considered to be precursor cells of the other epithelial cell types,
- Mucus-producing goblet cells (Fig. 2 D), and
- Fully differentiated ciliated cells, enabling the self-clearing mechanism of the airways.

Besides respiratory epithelial cells, the model also includes fibroblasts (Fig. 2 B), which functionally maintain the connective tissue underneath the epithelium and presumably are also involved in vitro in the formation of a basal membrane by intercellular communication with epithelial cells. Another special feature of our tissue model is the fact that it is based on a biological decellularized carrier structure which has already been



Natural human airway mucosa

3D tissue model



2 Comparison of natural human airway mucosa and the Fraunhofer 3D tissue model shows the high degree of similarity. Alzian blue staining is used to verify mucosa formation (blue).

successfully used in the clinical area for reconstruction of the tracheobronchial system in different patients. In the future, we are planning to use this tissue model also for the (further) development and testing of medical devices (e.g. airway stents) as well as for elementary infection studies with the causative agent of pertussis and parallel vaccine development.

In the lung carcinoma model, special substances can be added to simulate invasion via structures of the basal membrane into deeper layers of the matrix. This allows the testing of pharmaceutical agents also in advanced tumor stages. Our test systems can be used furthermore for identification of biomarker profiles, testing of combination therapies, and studies on how to overcome the development of resistances.



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### 3D lung carcinoma model

Similar to the colon carcinoma model, we have developed a 3D lung carcinoma model based on a decellularized matrix with different tumor cell lines. In practical use, treatment with TKIs (tyrosine kinase inhibitors) such as Gefitinib has already led to first successful results with tumors featuring an activating EGFR (epidermal growth factor receptor) mutation. We were able to demonstrate this effect also in our 3D model: Gefitinib displayed a significant effect specifically in cell lines with an activating EGFR mutation, and this effect was not observed in cell lines without this mutation. In a traditional 2D culture, the drug showed only a slight, non-significant effect. These findings suggest that the described 3D tumor test systems not only have higher chemoresistance than traditional 2D cultures, but can also enhance specific effects.



## IMPLEMENTATION OF THE 3-R PRINCIPLE

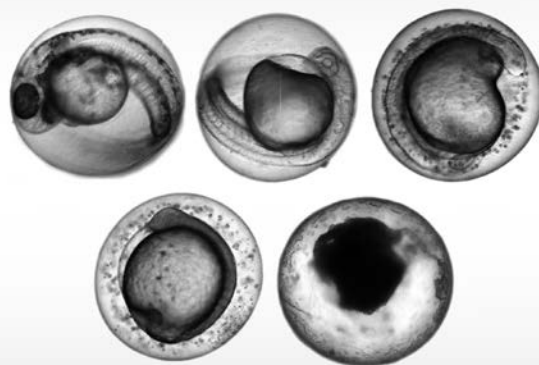
When Fraunhofer scientists develop new analytical methods, they apply the 3-R principle to Reduce, Refine, and Replace animal experiments. We have developed modern methods which do not involve animal tests. These methods allow many toxicological tests to be carried out not just in an ethically acceptable way but also more efficiently.

### **In-vitro cytotoxicity studies and immunotoxicological testing**

The immune system is one of the important target organs in the context of chemical exposure. Immunotoxicity can manifest either in the form of an overreaction (hypersensitivity, autoimmune reactions) or a reduced function (increased susceptibility to infectious agents, elevated cancer risk). Assessment of possible immunomodulatory properties of a substance is thus of pivotal importance and is, therefore, required by international regulations (EMA ICH S8, REACH). In line with the international trend towards reduction of animal experiments, we offer a comprehensive spectrum of in-vitro assays to test substances for cytotoxicity and immunotoxicity using established state-of-the-art methods. The xCELLigence real-time cell analysis system means that we possess an innovative method for ultrasensitive real-time analysis of cell growth and cell death with high time resolution. This system allows the impact of substances on cell functions (e.g. proliferation, adherence) and viability of adherent cells (e.g. epithelial cells, endothelial cells, macrophages) to be evaluated. Furthermore, different methods for immunotoxicological testing are available: the



1 Fish embryo screening  
(zebrafish).



1

effects of a substance on immune cell composition and function can be exactly characterized by multiparameter flow cytometry (immunophenotyping, intracellular detection of cytokines and phosphoepitopes). In addition, quantitative real-time RT-PCR (UPL principle) enables gene expression analyses of a wide range of immunologically relevant genes (cytokines, chemokines, pattern recognition receptors, defensins etc.). If need be, all cytotoxicity studies and immunotoxicological tests can be performed in compliance with GLP (certified GLP test facility).



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**Alternative ecotoxicological test methods**

The fish egg test with freshly fertilized eggs is recognized in the German Wastewater Ordinance as a preferred alternative to the acute fish test from an animal protection standpoint. The fish egg test is compatible with the requirement of REACH to reduce the number of tests on vertebrate animals. Fraunhofer scientists were involved in the development and validation of the test. At present, methods for bioaccumulation testing in non-vertebrates and cell systems are being worked out, in order to reduce bioaccumulation tests on fish.



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# SUSTAINABILITY





# SUSTAINABILITY AS A GUIDING THEME

## **How can we achieve the aim of “preserving for ourselves and our children a natural basis of life that is viable for the future”?**

The challenges we are currently facing are tremendous: climate change, water shortage, loss of biodiversity, soil degradation, and shortage of energy and raw materials. Pure relinquishment strategies are neither workable nor would they meet with public acceptance. Increasing ecological, economic, and social challenges require a system-oriented approach which promotes interdisciplinary thought and collaboration in order to develop innovative solutions. The basis is a perspective which integrates future requirements and demands with consideration of global justice. Research and development play a key role in achieving these goals. The focus is on innovative developments meeting the requirements of sustainable development. Future growth, taking into account the quality of life, must be achieved with only a fraction of our current resource consumption and a substantial reduction in emissions.

## **Sustainability is the word of the moment. But what exactly does it mean?**

The term was coined by Hans Carl von Carlowitz in a forestry context as early as 1713 in his book *“Sylvicultura oeconomica”*. This shows that mankind already recognized ecological limitations at an early point in time. An overall characterization of the term sustainability is provided in the definition framed by the Brundtland Commission (World Commission on Environment and Development – WECD) in 1987, which describes “sustainable development” as “development that meets the needs of the present without compromising the ability of future generations to meet their own needs”. Intragenerational as well as intergenerational justice is thus the key concept of

sustainable development. Sustainability is not a term that can be unambiguously defined in the sense of the natural sciences, but is rather a guiding principle of a normative nature. It describes a certain relationship between man and the environment which provides for the needs of both present and future generations.

The Fraunhofer-Gesellschaft’s commitment to contributing to sustainable development has already been enshrined in its mission statement: “The Fraunhofer-Gesellschaft supports efforts directed toward the sustainable development of society, industry, and the environment. The Fraunhofer institutes play an active part in such efforts through a responsible approach to the implementation of new technologies and through research and studies conducted on behalf of industrial and public-sector clients.”

Currently addressed sustainability topics cover: bio-based raw materials, raw material efficiency and resource management, water management, sustainable products and processes, climate change and cultural heritage, life cycle management and environmental assessment, renewable energy sources, energy efficiency and energy systems, and sustainable mobility.

We are implementing the sustainability concept in all our research activities; because sustainable development leads to innovation processes in industry and society and to the safeguarding of our natural basis of life.



## FRAUNHOFER GROUP FOR LIFE SCIENCES

The comprehensive and individually tailored services offered by the Fraunhofer Group for Life Sciences for the application of novel technologies require an organization that covers a broad range of disciplines, methods, and equipment. Under the motto “research for human health and the environment”, the Fraunhofer Group for Life Sciences offers its clients a rich pool of complementary expertise.

Six Fraunhofer institutes and a Fraunhofer research institution, each having proven in-depth expertise in different areas within the life sciences, are involved in this Group: the Fraunhofer institutes for Biomedical Engineering IBMT, Interfacial Engineering and Biotechnology IGB, Molecular Biology and Applied Ecology IME, Toxicology and Experimental Medicine ITEM, Process Engineering and Packaging IVV, Cell Therapy and Immunology IZI, and the Fraunhofer Research Institution for Marine Biotechnology EMB. Their combined knowledge of biology, chemistry, biochemistry, biotechnology, medicine, pharmacology, ecology, and nutritional science is thus pooled and synergized within this Fraunhofer Group. With the Fraunhofer EMB joining the Group in August 2012, marine biotechnology has become an additional focus. In all these Fraunhofer institutions, the scientists collaborate in interdisciplinary teams, so that tailored know-how concerning information technology, engineering science, and legal requirements is also available. Research and implementation at the client’s facilities therefore go hand in hand.

The Fraunhofer-Gesellschaft stands for reliable partnership in applied research. As the largest research organization of its kind in Europe, it develops market-oriented solutions tailored to the specific requirements of each client. A solid basis for this is its own pre-competitive research, geared to the basics and frequently undertaken in close cooperation with universities and other academic institutions.

One of the most important things we have learned: the path from the very first idea to the perfect solution is always very exciting – and we will gladly go down this path with you.

### Business units of the Fraunhofer Group for Life Sciences:

#### Medical Translational Research and Biomedical

**Technology:** The Challenge of Innovative Diagnostics and Personalized Therapy

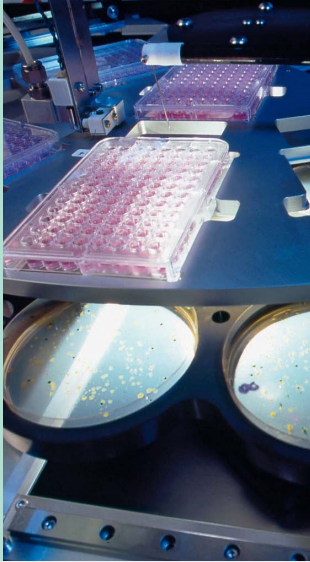
**Regenerative Medicine:** The Challenge of Qualified Biobanking and Controlled Self-Healing

**Healthy Foods:** The Challenge of High Consumer Acceptance and Disease Prevention

**The New Potential of Biotechnology:** The Challenge to Learn from Nature for Industrial Exploitation

**Process, Chemical, and Pesticide Safety:** The Challenge of Environmental and Consumer Protection





**Do you have any general questions regarding the Fraunhofer Group for Life Sciences, or any suggestions or requests?**

Dr. Claus-Dieter Kroggel, Head of the Group's Central Office, will be pleased to assist you, so that you can quickly reach your goal.

Prof. Dr. Rainer Fischer  
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