In our Business Unit “Registration and Risk Assessment”, we offer the studies and services required to assess the potential risks of chemicals to human health and the environment and to register these substances for the intended use. This concerns primarily (industrial) chemicals, biocides, and veterinary medicinal products. Main activities in this business unit include the preparation of registration documents on behalf of industrial clients and contract research for regulatory authorities and associations. For different substances and application areas, we also prepare toxicological expert reports, for example to assess residues or contaminations.

The focus is on the legal requirements valid for the substance in question and its intended use, and on the information necessary to meet these requirements. This information is compiled in cooperation with the client. Our broad range of services includes literature searches, identification of data gaps, and development of a testing strategy, including commissioning and monitoring of the required experimental studies. Furthermore, we determine human and environmental exposure data and compare them with the applicable limit values. All these steps are documented in the required registration and substance dossiers.

**Focuses of activity in 2014**

In 2014, a focus of activity in the Business Unit “Registration and Risk Assessment” was on improving chemical risk assessment, for example by means of integrated testing strategies (ITS). As an example, we are presenting in the below report two research projects conducted on read-across – a method consisting in extrapolating the toxicity of one or more source substances to the so far unknown toxicity of one or several target substances.

**Read across – further development of grouping concepts and their use in risk assessment**

In risk assessment, grouping approaches such as read across (RAX) are used to fill data gaps. This method consists in extrapolating the toxicity of one or more source substances to the so far unknown toxicity of a target substance, enabling both qualitative and quantitative predictions. RAX is used primarily for complex endpoints such as repeated-dose systemic and reproductive toxicity. For these endpoints, alternative methods such as in-vitro test systems or in-silico methods
By enhancing the above portfolio in close cooperation with our business units “Toxicology Testing” and “Environmental, Occupational and Consumer Protection”, we offer our clients a service tailored to their individual needs. The required studies can be performed at the Fraunhofer ITEM in compliance with international testing guidelines and with the principles of Good Laboratory Practice (GLP). Whenever necessary, we cooperate with other Fraunhofer institutes and also with external contract research institutions that have been our partners for many years.

In the future, alternative methods and tests without animal experiments and also integrated testing strategies shall be used increasingly in chemical risk assessment, so as to keep experimental studies to a minimum. To support this aim, we elaborate scientific basic principles in publicly funded projects and test their applicability in the regulatory context. In addition, our comprehensive activities in risk assessment frequently also spawn ideas for new scientific approaches that may help improve chemical risk assessment methodology in the future. For projects aimed at the development of alternative assessment concepts, such as the TTC concept, we have at our disposal comprehensive databases for toxicological endpoints from studies in rodents, which have been set up and further enhanced in this business unit over the past few years.

A great challenge of the RAX approach is demonstration not only of chemical but also biological similarity of the source and target substances. Biological similarity can be based on identical mechanisms of action, for example on an adverse-outcome pathway (AOP), or on similar degradation pathways involving the same critical metabolites and their kinetics. The comprehensive mechanistic data on toxicodynamics and substance metabolism (ADME) that are required for the evaluation of biological similarity, however, are in most cases not available for all substances of the RAX group.

Within different national and international projects and in close cooperation with partners, Fraunhofer ITEM scientists are currently developing new concepts for a better definition of “similarity” of the RAX compounds. The aim is to improve regulatory acceptance of the extrapolation by RAX and thus to contribute to a direct reduction of animal experiments. Two


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of these projects – one funded by the German Federal Ministry of Education and Research (BMBF) and one by the EU – will be described in the following.

**Example: BMBF project**

In the BMBF-funded joint research project “Entwicklung einer Strategie zur Bildung von Kategorien und Definitionen neuer Kategorien für die Endpunkte subakute, subchronische und chronische Toxizität zur Minimierung von Tierversuchen unter REACH” (development of a strategy for category building and definition of new categories for the endpoints sub-acute, sub-chronic and chronic toxicity to minimize animal experiments under REACH), a grouping concept was developed taking into account chemical similarity and toxicological profiles. The final data set, based on the RepDose and ELINCS databases, includes 1022 compounds, whose toxicity had been documented in in-vivo repeated-dose toxicity (RDT) studies. To achieve the best possible grouping of compounds, the following selection based on content and method was made:

- Toxicological endpoints: 28 organ-specific effects, grouped by thematic cohesion and chronology in repeated-dose studies with rats
- Toxicological potency: categorization of effect levels (LOELs) per endpoint by means of equal-frequency quantification
- Best method to deal with zeros: statistical imputation
- Structural information: SMILES codes for fragments and reactive groups
- Physicochemical parameters: selection of a few parameters with substantial contribution to the clustering
- Clustering method: predictive clustering tree (PCT)

Result of this project: A toxicologically meaningful grouping concept was achieved based on a data set refined by means of toxicological expertise and accompanied by manual verification of clusters during development of the model.

**Fraunhofer scientists appointed to committees**

Together with experts from federal and Länder authorities, universities, and other research institutions, experts of the Fraunhofer ITEM act as external, independent consultants to the German Federal Institute for Risk Assessment (BfR).

For the period 2014 to 2017, Dr. Annette Bitsch of the Fraunhofer ITEM has been reappointed to the BfR Committee for Food Additives, Flavorings and Processing Aids, and Dr. Katrin Schröder to the BfR Committee for Exposure Assessment and Exposure Standardization. A new appointment is that of Dr. Oliver Licht, who is now a member the BfR Committee for Contaminants and other Undesirable Substances in the Food Chain.

"Ensemble of Classifier Chains“ turned out to be the best method for model development (www.ml-reach.informatik.uni-mainz.de).

**Example: EU project DETECTIVE**

The aim of the EU-funded project DETECTIVE of the SEURAT research cluster is to enhance the existing knowledge about mechanistic processes associated with toxic events in man. Predictive biomarkers are being developed to this end, e.g. by combining different "omics" technologies. Before they can be used in risk assessment, these biomarkers, representing key or intermediate steps in such mechanisms, have to be validated. A concept for this approach is currently being developed by means of a RAX case study with valproic acid (VPA).
In 2014, Dr. Kathrin Schröder, Dr. Annette Bitsch and Dr. Oliver Licht were appointed as independent consultants to different committees of the German Federal Institute for Risk Assessment.

First of all, candidate biomarkers were identified in the TG-Gates database, based on transcriptome alterations associated with lipid and energy metabolism. According to this database, VPA is a potent inducer of such transcriptomic alterations. In addition, a causal relationship with effects observed in RDT studies could be established. VPA induced effects such as fatty liver, lipidosis, and vacuolization of hepatocytes after oral exposure.

To evaluate predictivity of the biomarker candidates, ten substances with similar structures, consisting in (un-)branched carboxylic acids for which RDT studies were also available, were identified. In-vivo data were obtained from the databases RepDose, IMI eTOX, ECHA CHEM, Cosmos, Leadscope and Nedo, and also from literature. Based on the effects observed in these studies, four VPA analogues were classified as “in vivo positive” and five as “in vivo negative”. One analogue was defined as “borderline case”, because the observed effects did not allow an unambiguous conclusion about deregulation of the lipid metabolism. At present, the biomarkers are being evaluated in vitro in different cells by quantitative RT-PCR: in human primary and rat hepatocytes and in HepG2 cells. The aim of this RAX case study is to develop an integration concept for the resulting biomarkers – qualitative markers to be used in a RAX approach and quantitative markers for use in chemical risk assessment without taking into account structurally related compounds.

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Equipment highlights

Databases
- RepDose (containing data on repeated-dose toxicity of chemicals)
- FeDTex (containing data on developmental and reproductive toxicity of chemicals)
- PaFtox (containing data on repeated-dose toxicity of nanoparticles)

Models
- Modeling software for human and environmental exposure assessment

Documentation
- Literature management with over 100,000 entries in 500 subject areas, with searching possibilities and access to 150 journals