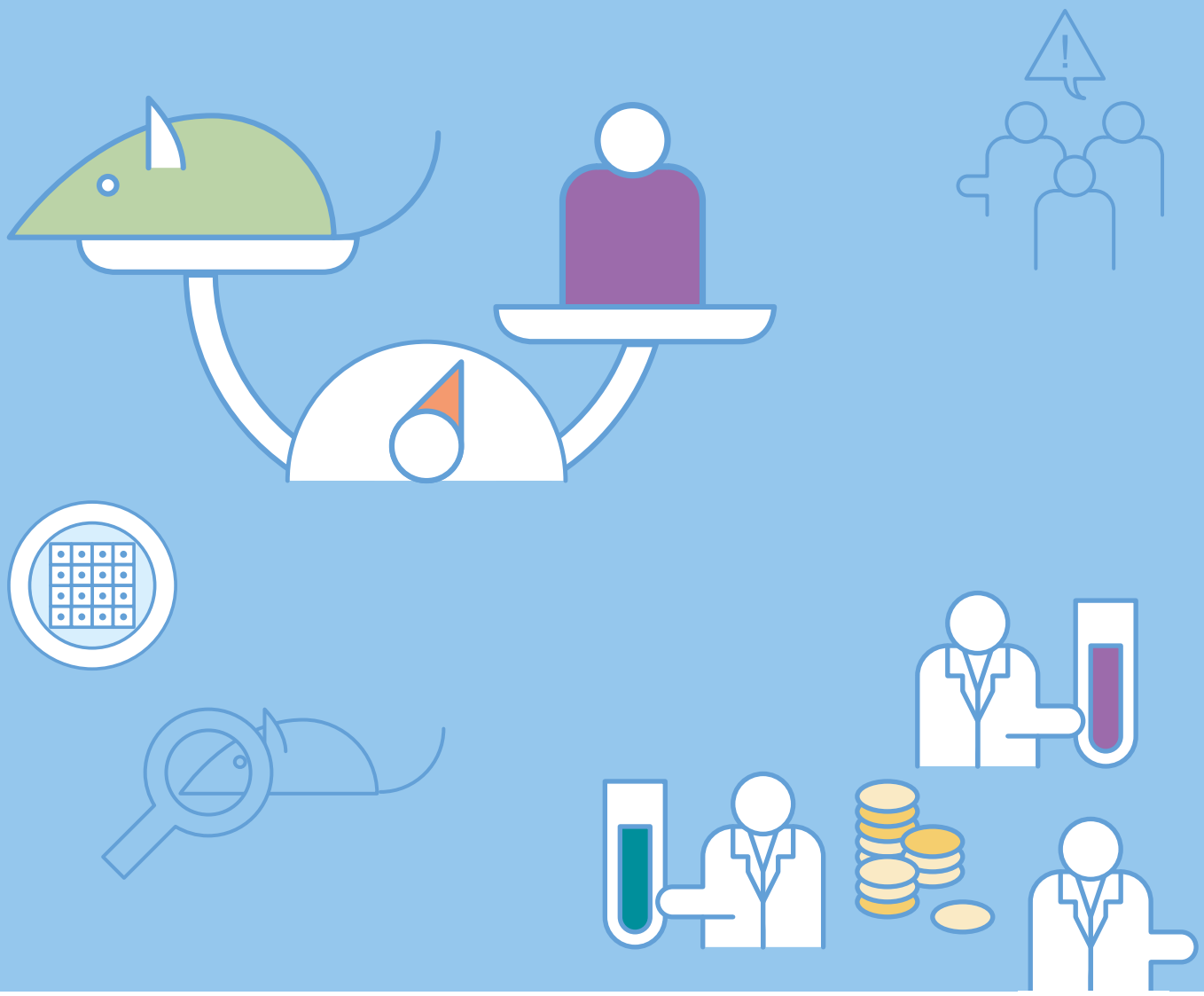
Protection of Laboratory Animals

Genome data comparisons for identifying suitable animal models



DEUTSCHES ZENTRUM
ZUM SCHUTZ VON
VERSUCHSTIEREN

BfR
Bundesinstitut für Risikobewertung

The German Centre for the Protection of Laboratory Animals (Bf3R) at the BfR

The Centre combines the various areas of alternative method research on a national level in line with the 3R principle. The Centre coordinates activities all over Germany with the goals of restricting experiments with animals to a level which is absolutely necessary and affording laboratory animals the best possible protection. In addition, impetus is to be given to national and international research activities through the work of the Centre while encouraging scientific dialogue at the same time. Bf3R was established in 2015 in the course of the Animal Welfare Initiative of the Federal Ministry of Food and Agriculture. It is an integral component of the BfR which is subdivided into five areas of competence.

www.bfr.bund.de
> German Centre for the Protection of Laboratory Animals

Imprint
Printed extract from the BfR Science Magazine BfR2GO, Issue 1/2017, p. 38–43

Publisher:	German Federal Institute for Risk Assessment
Edited by:	BfR Press and Public Relations Unit
Photos:	neues handeln: Cover; BfR: p. 7
Illustrationen:	Janine Czichy: p. 3, 4, 6; Studio Good: p. 7
Layout:	Studio GOOD, Berlin
Print:	Druck- und Verlagshaus Zarbock GmbH & Co. KG, Frankfurt am Main
Translation:	ABC Sprachschule und Übersetzungsbüro, Niederkassel-Mondorf
Copies printed:	250 (German), 250 (English)

This text version is a translation of the original German text which is the only legally binding version.

BfR2GO 1/2017
ISSN 2567-8949 (Print)
ISSN 2567-8957 (Online)

BfR2GO is published twice a year.
Free subscription www.bfr.bund.de/en

Wanted: mirror image

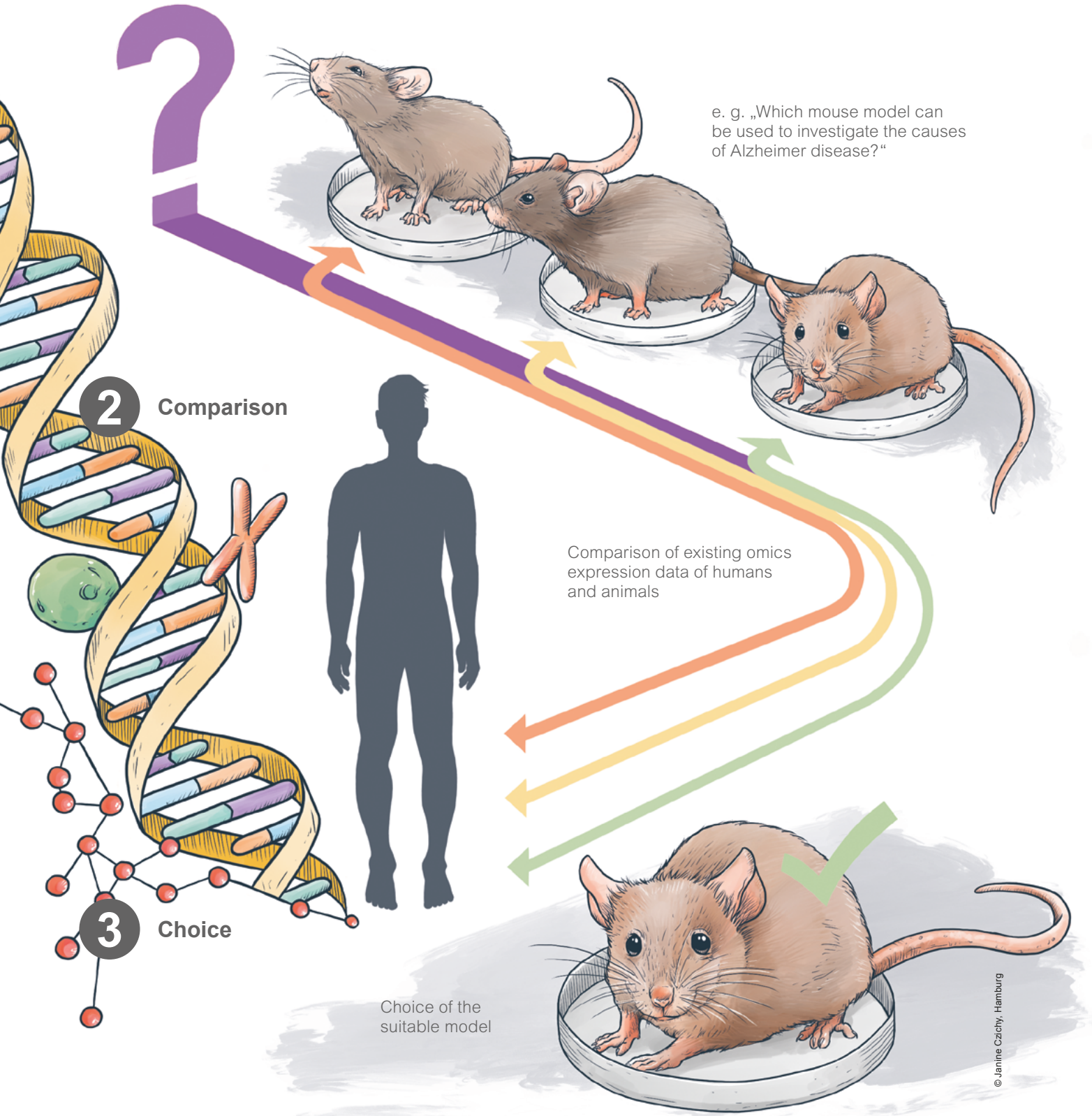
New approach to genome data comparisons of humans and animals

The German Centre for the Protection of Laboratory Animals (Bf3R) at the BfR indicates a new approach in order to identify the suitable animal model for basic research or specific clinical questions with the help of gene expression data. This means that it will be possible to avoid unnecessary animal experiments in future. Hamburg's scientific authorities presented a research award to the Centre in recognition of the insights gained during this process.

Is the course of an inflammatory process in mice similar to that in humans? Are mice therefore suitable for use in experiments to test anti-inflammatory substances? Scientific questions are often investigated in animal experiments in order to transfer research findings from basic research into daily medical practice – this is the field known as translational research. Whether and to what extent the results also apply to humans is regularly the subject of controversial debate. Suitable methods that scientists could use to select animal models that are fully transferrable to humans would underpin scientific progress and help to reduce the number of animal experiments. The key question in this respect is which animal model best reflects the "human system".

Identifying suitable animal models

Animal models are used to investigate numerous biological processes and diseases such as wound healing, diabetes, tumours or inflammatory diseases. Comparison of the omics expression data in animal models and humans can help to identify suitable models for the issue in question.



Comparison of genome data prone to error

These considerations are based on data from modern, bioanalytical high-throughput techniques. The scientific community is increasingly using these so-called omics technologies that analyse the entirety of all genes and their products in both animals and humans. These technologies can comprehensively depict molecular processes in living systems – by showing the interaction of hundreds to thousands of genetic products, for example. However, as the corresponding procedure is not standardised, the analysis of this increasingly complex omics data sets presents major challenges for scientists. To date, the interpretation of the findings has mostly depended on the expertise of the scientist in question and the method used. As a result, the decision on the question of the transferability of findings to humans was prone to error, and this made the search for suitable animal models more difficult. The low level of standardisation with regard to the interpretation of omics data has already led to contradictory results in research circles; this was the case with two studies conducted in 2013 and 2015 focusing on the comparability of inflammatory diseases in humans and standard mouse models. While one research group concluded in 2013 that inflammatory processes in humans and mice are not comparable, another group reached the opposite conclusion two years later based on the same omics data, claiming that mice react very similarly to humans on the molecular level and that they are extremely useful as animal models for investigating human diseases. Although it is not unusual for experts to differ in their assessment of the findings of studies, this is seldom the case when the underlying data is identical.

The motivation of the German Centre for the Protection of Laboratory Animals (Bf3R) at the BfR was to clarify these contradictions and develop a standardised and targeted analytical approach that simplifies the search for suitable animal models and therefore helps to avoid unnecessary animal experiments. One of the core tasks of the Bf3R is to coordinate all activities nationwide with the aim of limiting animal experiments to those that are absolutely essential and ensuring the best possible protection for laboratory animals. The tasks of the Bf3R are performed by the BfR.

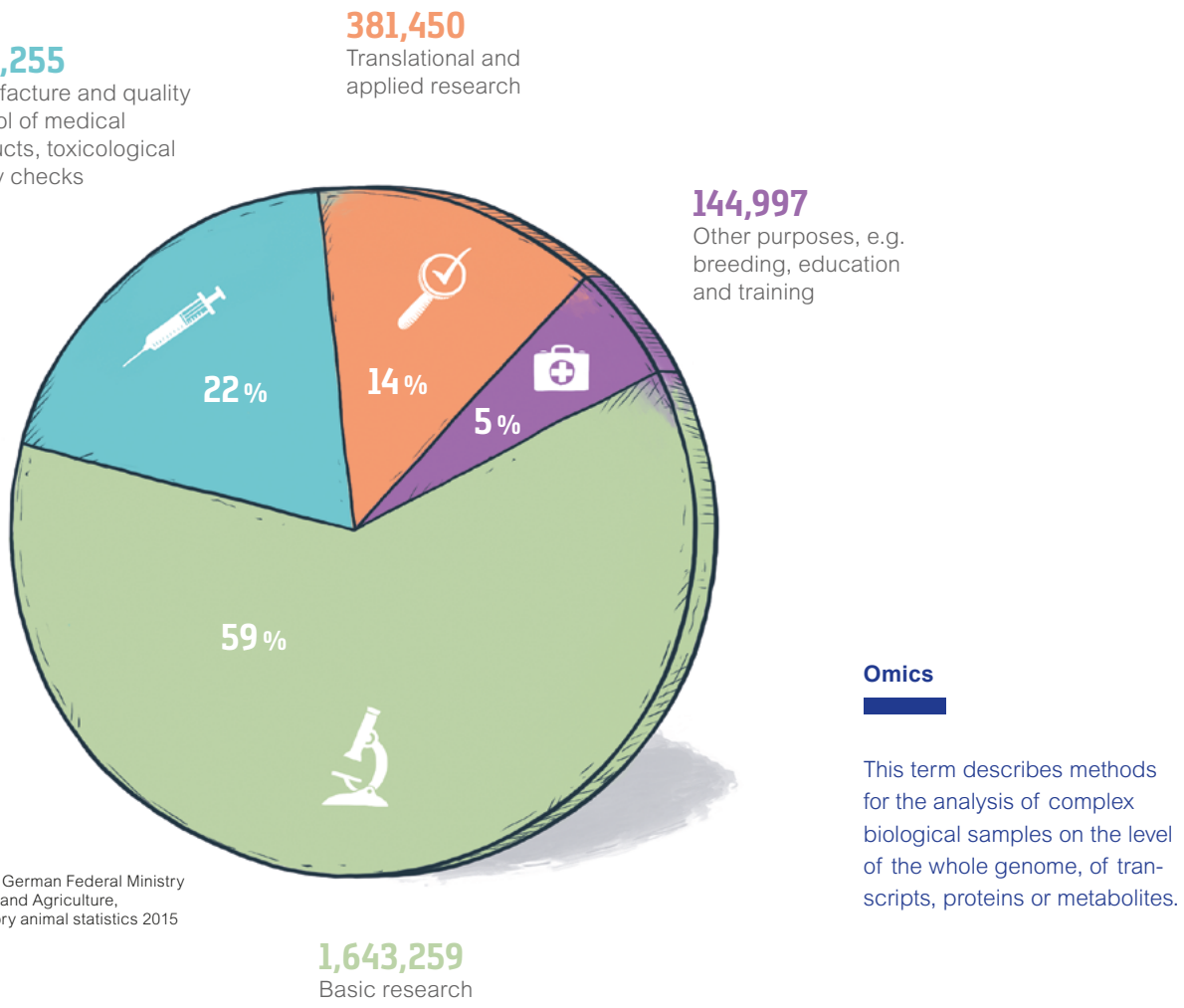
Targeted comparison of animal and human genome data

The omics data from the two contradictory studies mentioned was systematically evaluated again using powerful computers by the scientists of the Bf3R. In this process, the genes to be compared were first assigned in groups to biological processes that are essential for an inflammatory process. This method, known as gene set enrichment analysis (GSEA), uses comprehensive genome data of common animal models and of patients stored in public databases. With their approach, the scientists of the BfR were then able to detect changes at the level of pathological signal pathways and identify differences between animal models and humans. Because the majority of the gene products evaluated were only changed slightly, it was possible to include all genes in the analysis at the level of complete biological signal pathways and evaluate changes in a biological signal pathway as a whole. This represents a significant difference as compared to previous analysis methods because, until now, genes from humans and animal models were often selected arbitrarily and subjectively for a direct comparison in order to address translational questions. The Bf3R's systematic evaluation showed that the results of this genome data comparison for certain mouse models were consistent with the data determined for humans. For other mouse models, on the other hand, this was not the case. For example, the inflammatory process in mice that are infected with *Staphylococcus aureus* (*Staphylococcus aureus* injection model) or are subjected to intestinal perforation (cecal ligation and puncture model) is similar to most clinical samples. In contrast, diseases caused by lipopolysaccharides (LPS) and *Streptococcus pneumoniae* show a different course in mice than in humans.

Research approach helps to reduce animal experiments

The GSEA approach to data analysis will make it easier for research groups to select the optimum animal model most closely mirroring the human situation in a targeted and standardised manner in the future. A prerequisite for this is the existence of omics data relating to the clinical question and to corresponding animal experiments. This is already the case for a large number of animal models and human illnesses, including not only inflammatory diseases but also, for example, cardiovascular diseases, cancers, respiratory diseases, metabolic disorders and neurological diseases. Due to the increasing use of omics methods, it can be assumed that additional systems biology data will be steadily acquired and published. For this reason, the method applied by Bf3R will continue to be useful in the targeted selection of animal models. This method can be used in basic research as well as translational and applied research. In 2015, according to the German Federal Ministry of Food and Agriculture, the majority of animal experiments (a total of 73 %) took place in these research fields. Many of these experiments were performed on mice, rats, and, increasingly, fish. In principle, the new method can be used to select the suitable laboratory animal model and/or exclude unsuitable animal models for all animal experiments in these fields. Similar to the numbers for animal experiments, most omics data is currently available for mice and rats. For example, data is listed for approximately 340,000 mouse samples and 75,000 rat samples in the public NCBI/GEO (National Center for Biotechnology Information/Gene Expression Omnibus) database alone.

Purposes for which laboratory animals are used in Germany



Source: German Federal Ministry of Food and Agriculture, laboratory animal statistics 2015

Omics

This term describes methods for the analysis of complex biological samples on the level of the whole genome, of transcripts, proteins or metabolites.

Method identifies suitable cell culture models

The Bf3R's method is not restricted to human and murine data sets, but can be applied to all other species for which extensive databases on biological signal pathways or gene groups exist. The new research method can also be used for the evaluation of cell-based alternative methods. A systems biology comparison of the clinical data with omics data from, for example, modern 3D cell cultures or organ-like microstructures enables the characterisation and verification of cell-based alternative methods.

Award-winning research work

In 2016, the scientists of the BfR received the Hamburg Research Award for promoting the development of alternative and complementary methods. The research prize with a value of €20,000 was awarded for the first time by the Hamburg Agency for Health and Consumer Protection and the Hamburg Agency for Science, Research and Gender Equality. The prize is awarded for work that contributes towards replacing or minimising animal experiments. ◘

More information:

Weidner et al. 2016. Defining the optimal animal model for translational research using gene set enrichment analysis. EMBO Molecular Medicine 8: 8, 831–838.

Weidner et al. 2017. A protocol for using gene set enrichment analysis to identify the appropriate animal model for translational research. J Vis Exp. Aug 16;(126). DOI: 10.3791/55768.

Spectrum

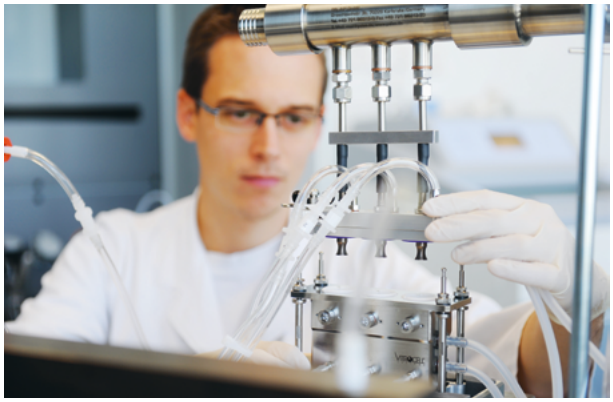
Alternatives to animal experiments: pooling regional research

Individualised pain therapy for laboratory mice and a reduction in animal experiments for *testing the inhalation toxicity of nanomaterials* (see column on the right) – the BfR's initial work results in the Berlin-Brandenburg cooperative project BB3R will contribute to these topics in the future. The network of universities and federal institutes performs research on avoiding animal experiments (replacement) or reducing their scope (reduction) and on decreasing the suffering of laboratory animals (refinement). As one of six project partners, the BfR is involved in the refinement and replacement research fields. The German Federal Ministry of Education and Research is supporting the project, abbreviated to BB3R, for four years until the spring of 2018; it is designed as a platform for scientific communication and includes a graduate education programme.

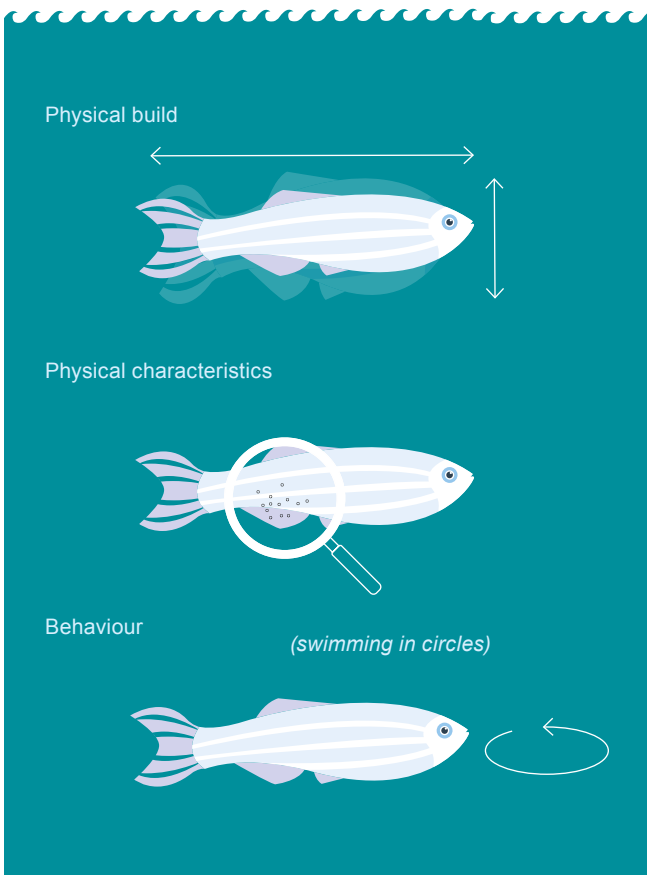
More information:
www.bfr.bund.de/en > Research
www.bb3r.de/en

Testing the inhalation toxicity of nanomaterials

Inhalation is considered the most significant path of entry for nanomaterials. In the context of BB3R research, the BfR is reproducing this type of exposure using *in vitro* aspiration epithelium models at their interface to the air. This approach to testing inhalation toxicity helps to reduce the number and scope of animal experiments.



Test setup: three tubes conduct a test aerosol with airborne nanomaterials to epithelial cells.



Determining suffering of laboratory fish

The question of whether laboratory fish feel pain or suffer can be answered based on specific criteria. Together with external experts and representatives of approval authorities, the "National Committee for the Protection of Animals Used for Scientific Purposes" at the BfR coordinated the preliminary work on this topic. The criteria, defined for the first time, evaluate anomalies in the physical build and behaviour of the animals, for example. In this way, pain and suffering can be treated and alleviated in a targeted manner. Because the criteria clearly address the question of the animals' distress, they also aid decision making for authorities responsible for approving the breeding of genetically modified animals that could experience pain or suffering. After mice and rats, fish (particularly zebrafish) represent the third most frequently used laboratory animal type. The number of laboratory fish has increased steadily over the past few years.

More information:
Bert et al. 2016. Considerations for a European animal welfare standard to evaluate adverse phenotypes in teleost fish. EMBO J. 35: 11, 1151–1154.
Recommendations of the National Committee TierSchG Nr. 001/2015

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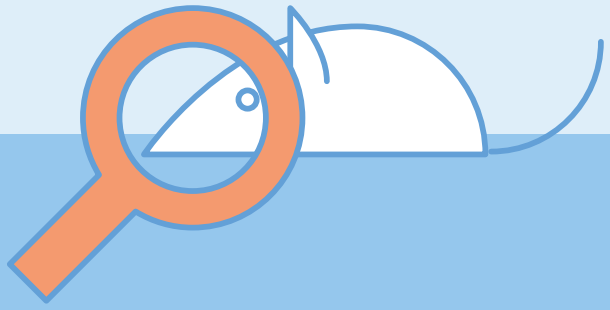

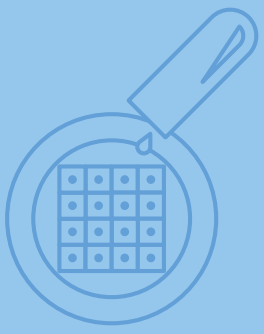

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