

Searching for Literature and Information on Alternative Methods

Barbara Grune

Federal Institute for Risk Assessment

ZEBET-Database and Information Services

Animal Welfare Regulations



Council Directive 86/609/EEC (24 November 1986) ... regarding the protection of animals used for experimental and other scientific purposes,

Replacement Alternatives - Article 7(2)

“Animal experiments shall not be performed if scientifically satisfactory methods of obtaining the result sought, not entailing the use of an animal, are reasonable and practicably available.”

Reduction and Refinement Alternatives - Article 7(3)

“The choice of species shall be carefully considered. Methods shall be selected that use the minimum number of animals, involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress or lasting harm.”

Refinement Alternatives - Article 7(4)

“All experiments shall be designed to avoid distress and unnecessary pain and suffering to the experimental animals.”

Proposal for a Directive of the European Parliament and of the Council on the Protection of Animals used for Scientific Purposes, 5 November 2008

The aim of a search

1. Survey

(to get an idea of a topic, find suggestions etc.)

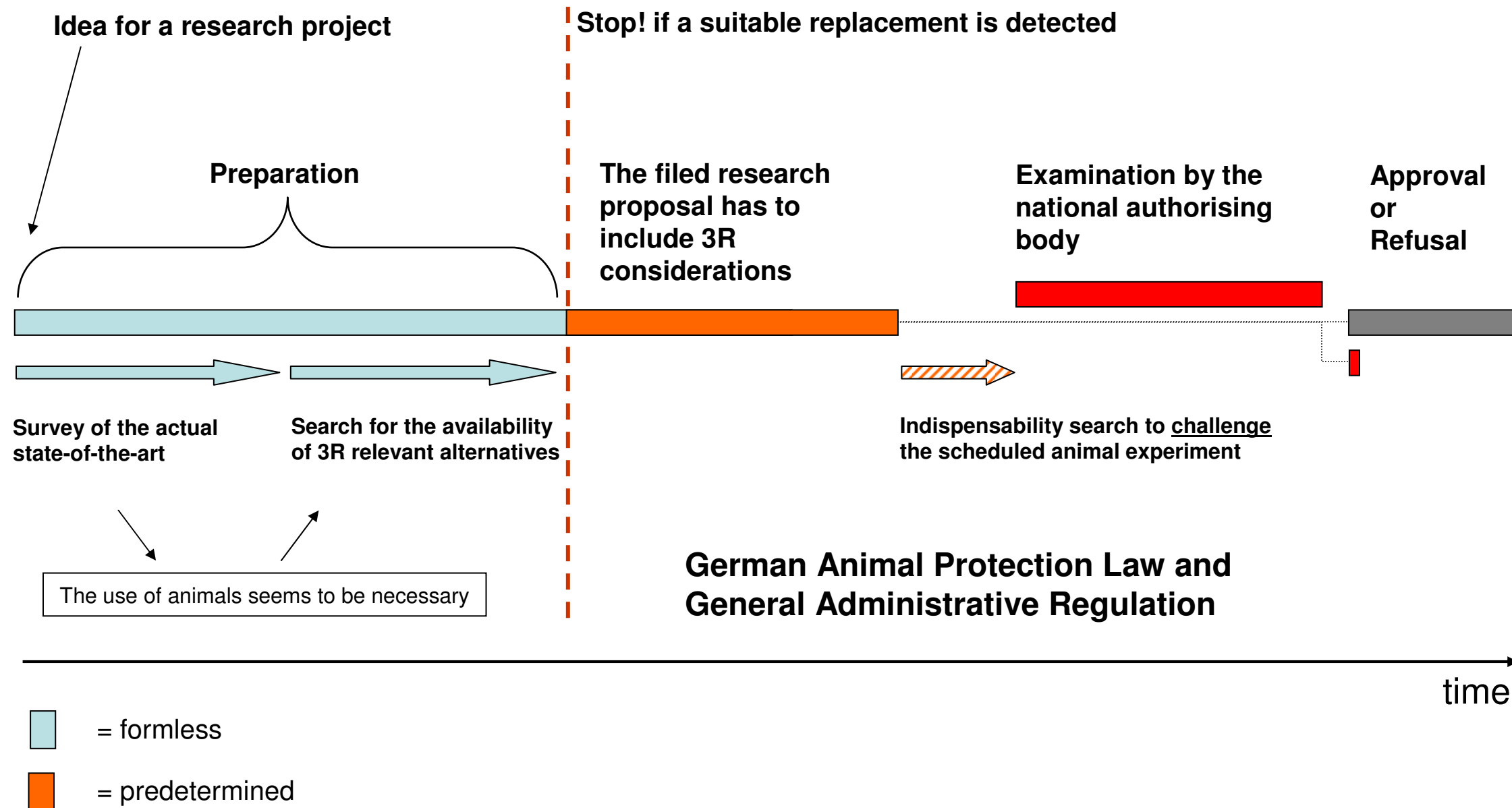
The aim of a search

2. Targeted Search (structured) (to target specific information or to exclude the presence of a particular piece of information; indispensability search)

German animal protection law § 7 (2)

„.... examine, whether the pursued purpose cannot be achieved by other methods or procedures “

Search-stages during the planing of an animal experiment



The Challenge: Searching Information on Alternative Methods

Where to find information? - Chose the appropriate information resources

Millions of potentially relevant documents are scattered across the Internet

databases (literature, factual, patent, added value) / database hosts
open access resources / organizations and their services / intranet

How to find information? - Compile relevant und necessary search terms

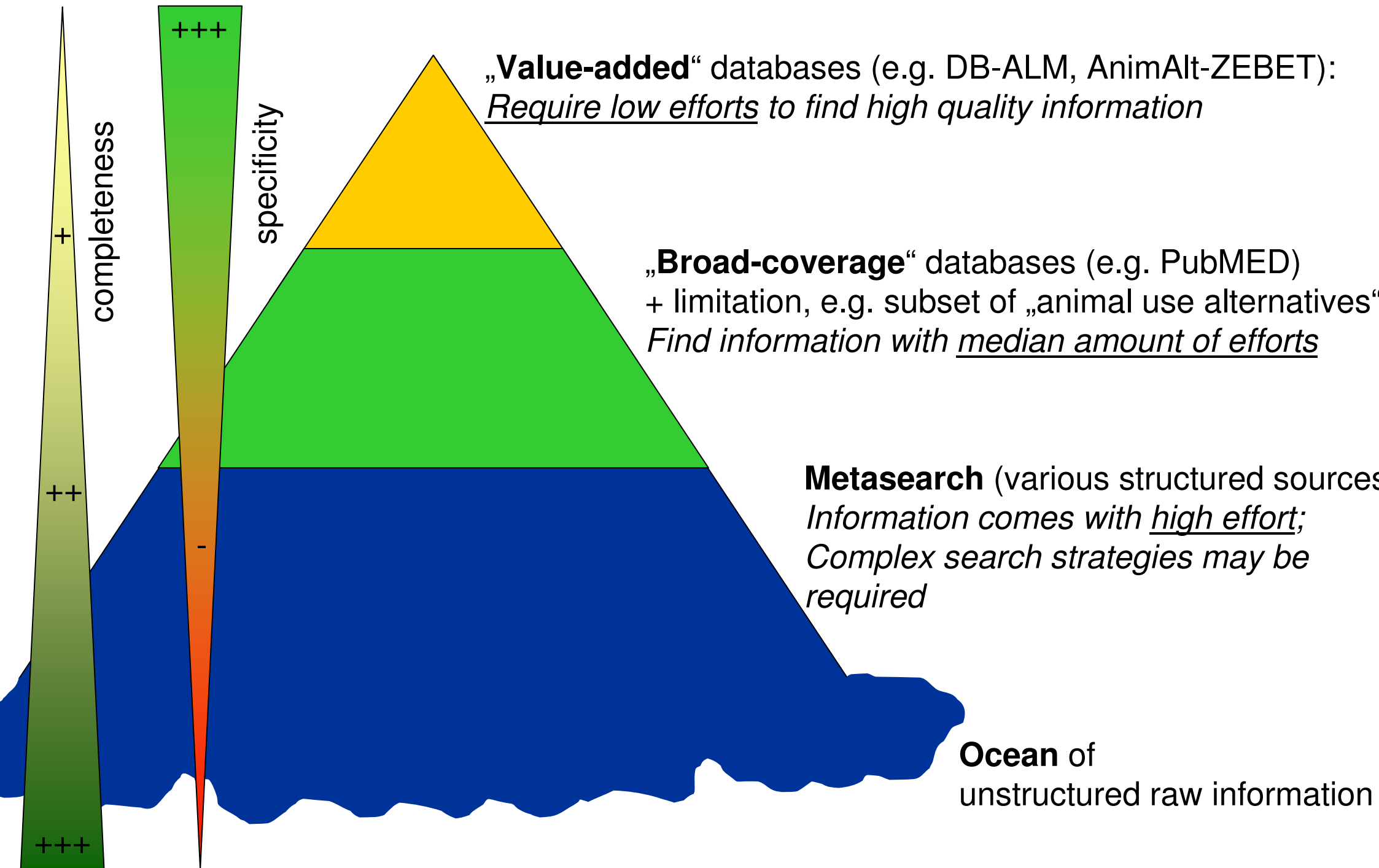
search terms (linguistic variations, synonyms)
search operators

Quality of a search result

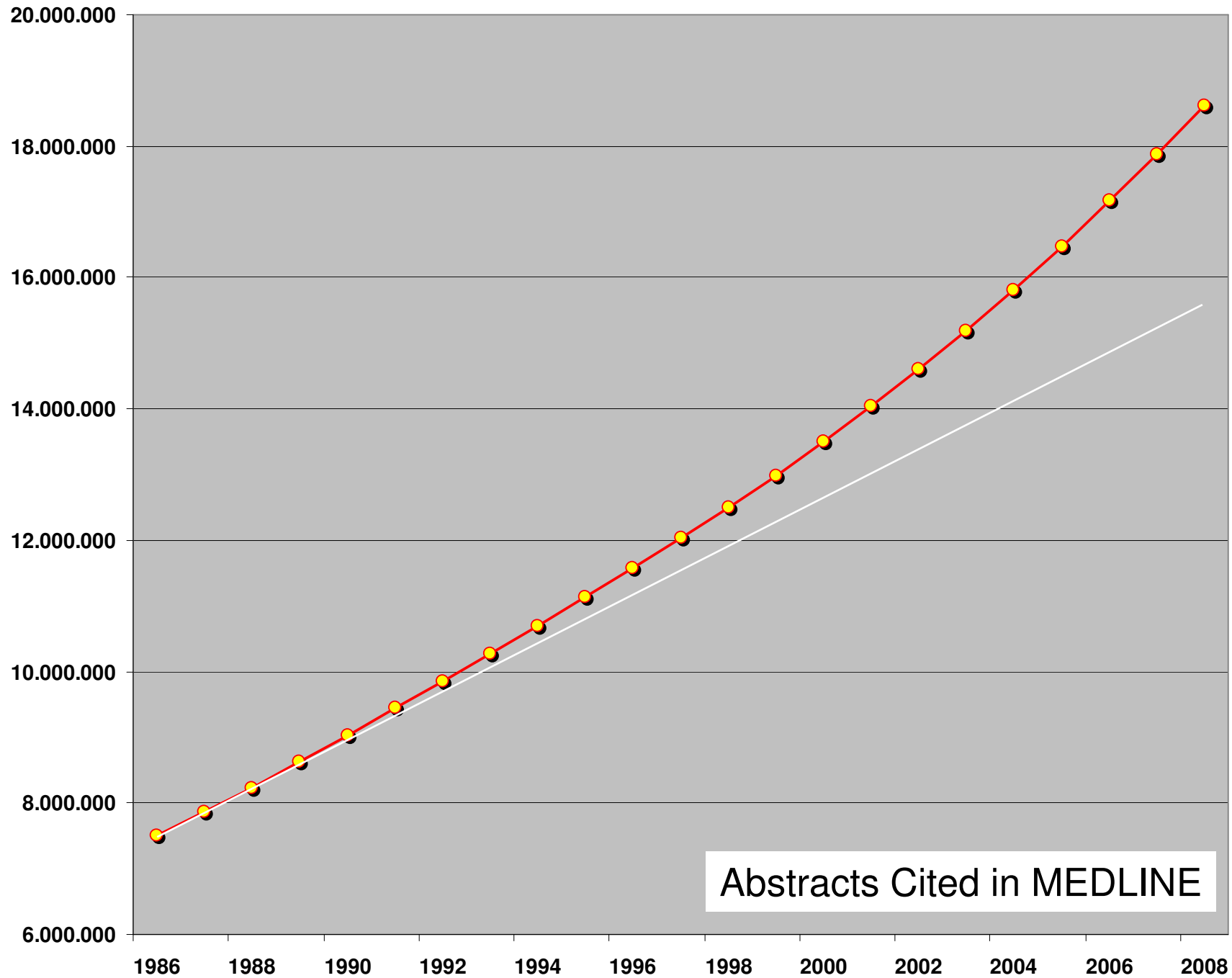
Completeness (recall) / Relevance (precision) /
Currentness / Reliability (peer reviewed)

Information can be a basis for decision-making

Proposal for a 3R-specific search routine



State of the scientific knowledge

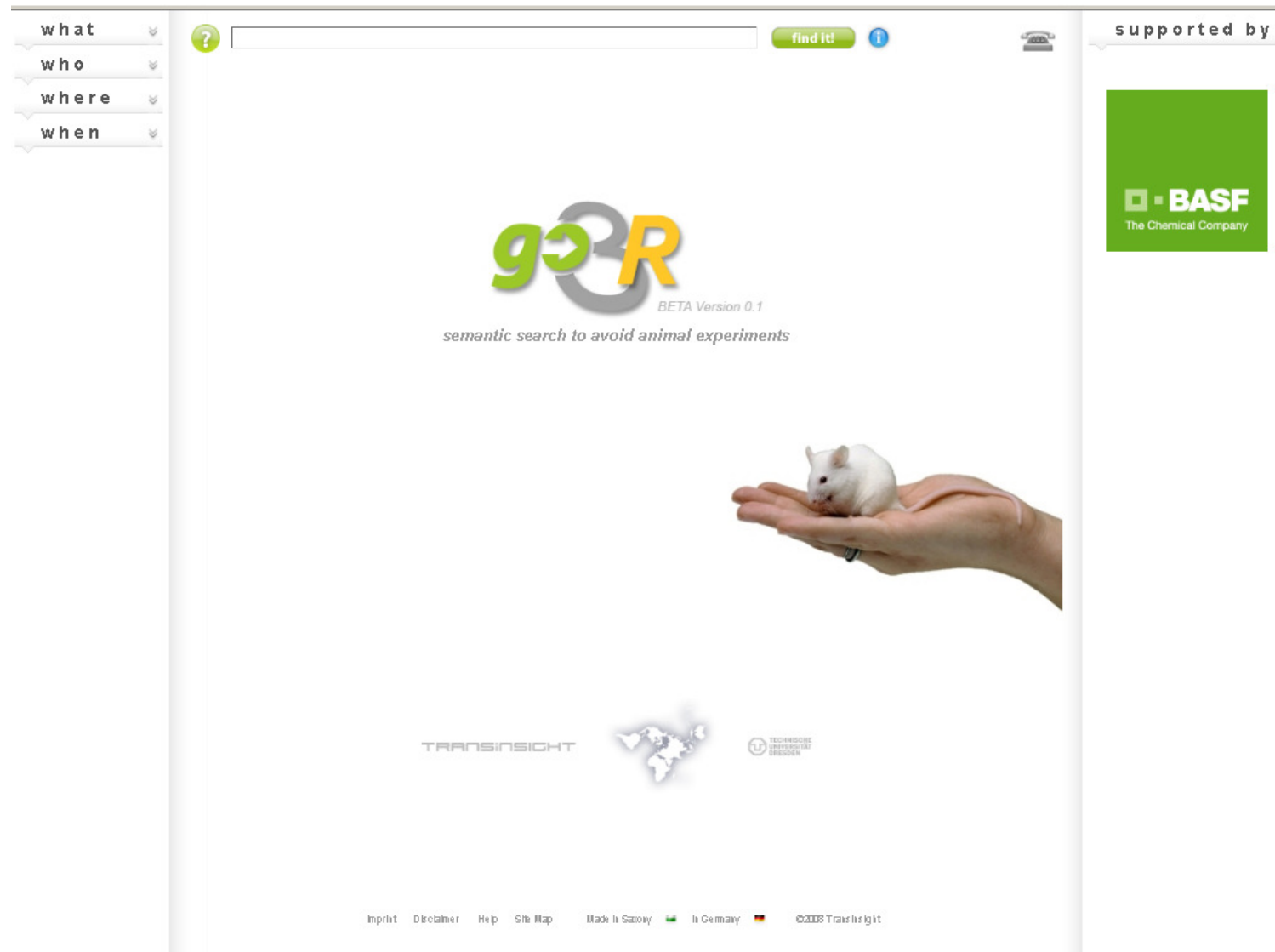


Quantity vs. Quality: How to get both?

The main problem is not quantity, it is quality!

Our Answer: Semantic Technologies

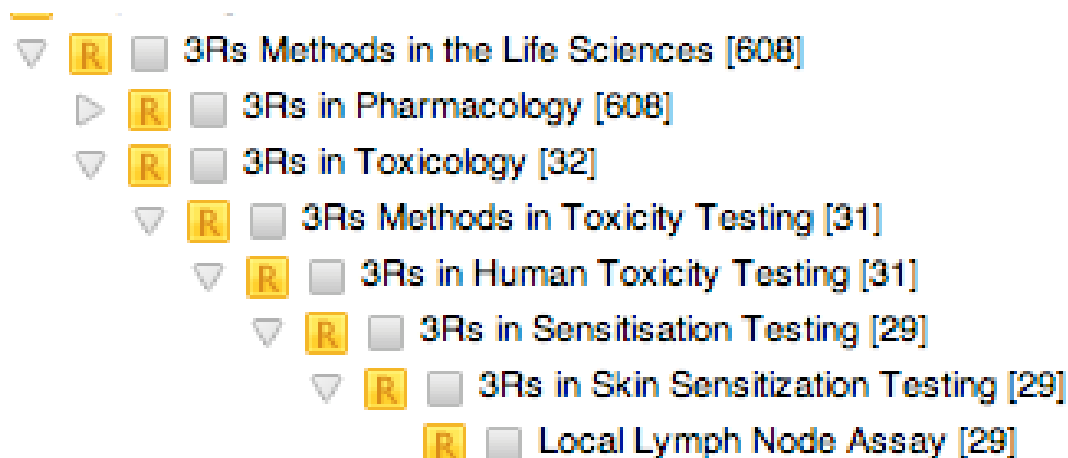
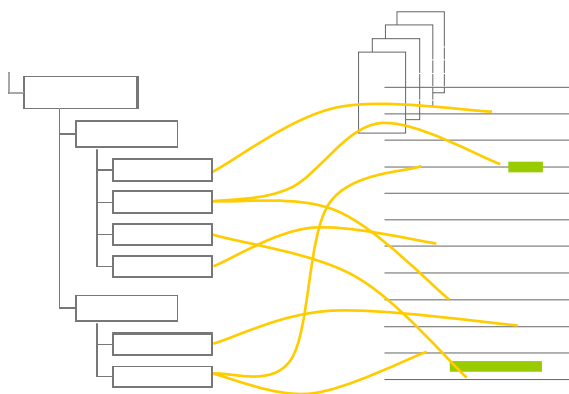
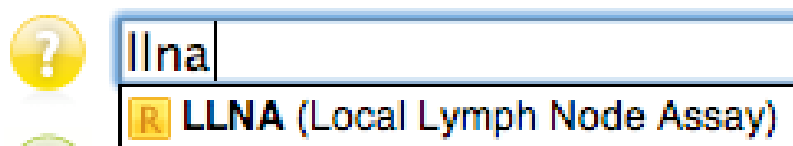
<http://www.Go3R.org>



How does it work? Handling ambiguities

- **BCOP** is a Charitable organisation dedicated to improving the quality of life
- Scale-invariant feature transform (or **SIFT**) is an algorithm in computer vision
- **AMES** successfully educates and services over 40000 people every year
- **LLNA** Lucca Leadership North America

Go3R tries to understand your query:



Next Steps

Expansion of the Go3R ontology

Go3R ontology (October 2009)

26 branches comprise 16.998 concepts
e.g. 3Rs Institutions
3Rs Methods in the Life Sciences
Animal Care and Handling



TRANSINSIGHT



Scientific Consultancy –
Animal Welfare
Neubiberg/Munich

„Nano-branch“ of Go3R (October 2009)

8 nanobranches comprise 1.361 concepts enriched with 2.628 synonyms
e.g. Endpoints for Nanomaterial Characterisation & Testing
Methods for Nanomaterial Characterisation & Testing
Nanosubstances, Nanoproducts, etc.

The AnimAlt-ZEBET Database,

***A Unique Resource for Comprehensive
and Added-Value Information on 3R Alternatives***

Daniel Butzke

Federal Institute for Risk Assessment

ZEBET-Database and Information Services

AnimAlt-ZEBET database is ...

... a **reliable resource** for information on **alternatives to all kind of animal experiments**, that presents the information in an **user oriented** and **easy-to-follow** fashion and **supports scientists, authorising bodies** and others who have to consider the indispensability of a planned animal experiment **with all the available expertise**.

- online since 2000
- free of charge
- currently hosted by DIMDI (www.dimdi.de)



AnimAlt-ZEBET, stand-alone version online available soon

ZEBET - Alternatives to Animal Experiments
Methods und Literature

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User signed on: Dr. Butzke, Daniel

AnimAlt-ZEBET

Database for Alternative Methods to Animal Experiments of the Centre for Documentation and Evaluation of Alternatives to Animal Experiments (ZEBET)



In the ZEBET database only those methods are documented which fulfil at least one of the three following criteria:

- the method can be used to replace animal experiments (Replacement)
- the number of experimental animals is reduced (Reduction)
- the pain and suffering of the experimental animals are minimised (Refinement)

These criteria correspond to the scientific principle of the 3Rs for alternative methods to animal experiments which was published by W.M.S. Russel and R.L. Burch in their book "The Principles of Humane Experimental Techniques" in 1959.

AnimAlt-ZEBET provides reports containing a scientifically evaluation of the alternative methods by ZEBET's experts with regard to their contribution to the 3Rs principle.

Welcome to the ZEBET-Database for Alternatives to Animal Experiments!



Browse through currently ~140 method summaries

ZEBET - Alternatives to Animal Experiments
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Browse through currently ~140 method summaries

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| <i>Food Hygiene</i> | 5 |
| <i>Cell Biology</i> | 1 |
| <i>Animal Production</i> | 2 |

Place more emphasis on alternatives in basic research



„Humanised“ mice:

- for the generation of therapeutic „human“ antibodies
- suitable **alternative**:

„Phage Display“ - technique

e.g. Adalimumab (Humira) for treatment of arthritis

GFP-transgenic pigs

taxodros.unizh.ch

Transgenic flies as model of
Alzheimer's disease

Aasen et al Nat Biotech 2008

Key information at a glance

| Methoden | |
|-------------------|---|
| Method No. | 25 |
| Last Revision | 27.03.2009 |
| Section Heading | Toxicology |
| Title | The Hen's Egg Test - Chorio-Allantoic Membrane (HET-CAM) in vivo assay for identification of Ocular Corrosives and Severe Irritants (see AnimAlt-ZEBET-method 324 for its application to identify non-irritants) |
| Terms | BGA classification model; Draize test; GHS; HET-CAM; IS; ITC; ITS; OECD TG 405; R41; SDS; ZEBET; animal experiments; animal testing; coagulation; conjunctival damage; haemorrhage; hen's egg test; integration; concentration; ocular lesions; ocular irritation; test guideline 405; tiered testing system; chicken; chorio-allantoic membrane; eye irritation; globally harmonized system; irritants; irritation score; irritation threshold; rabbit eye test; rabbits; reduction; surfactants; vascular lysis |
| Evaluation | Replacement |
| Status | Regulatory Acceptance |
| Regulation | EU (European Chemicals Bureau): Manual of decisions for implementation of the sixth and seventh amendments to directive 67/548/EEC on dangerous substances (Directives 79/831/EEC and 92/32/EEC) |
| Abstract | <p>3R RELEVANCE</p> <p>Currently, the HET-CAM assay is an approved in vitro method to detect severe eye irritants in the European Union only. When a positive result is obtained, the substance will be classified and labelled accordingly with no further in vivo testing. Negative results require subsequent in vivo testing to distinguish between weak eye irritants and non-irritants.</p> <p>The assay is considered amenable for application of a broad range of solid and liquid substances with only few limitations. Special protocols and prediction models for certain substances (surfactants; substances with low solubility etc.) are available.</p> <p>BACKGROUND</p> <p>Since June 2007 the European REACH regulation (1907/2006/EC) requires that all substances</p> |

New structure of the Abstract

3R Relevance: *the essential facts*

Background

- **Scope:** *in what context is the experiment required*
- **Animal experiment:** *description of the substituted animal experiment*
- **Alternative approaches:** *comparison of existing alternatives*

Method

- **Principle:** *how does it work*
- **Procedure:** *the essentials of the most elaborate (recommended) protocol, and information on the required equipment*
- **Prediction Model:** *what is the output*

Expert Panel Opinions

wording of ESAC statements, ICCVAM evaluation reports etc. in extracts

Note

application domain, limitations, performance standards, variations of the basic protocol etc.

BACKGROUND

Since June 2007 the European REACH regulation (1907/2006/EC) requires that all substances manufactured in the EU or imported at an amount of 1 tonne or higher are tested for health and safety impairments. REACH also requires that substances of very high concern* for human health or the environment, regardless of their distribution, safety testing also is obligatory for plant protection (91/414/EEC), biocidal products (98/8/EC), and cosmetics (76/768/EEC).

The European Council Regulation No 440/2008 (the "Test Methods Regulation") and a set of OECD guidelines specify of how the potential harm inflicted by a chemical must be characterised. To date, there is only one adopted OECD guideline (TG 406) that specifies characterization of the "acute eye irritation/corrosion" potential of a chemical. This guideline describes the in vivo Draize test with albino rabbits. When a chemical induces tissue damage to the eye or serious physical decay of vision, which is not fully reversible within 21 days of application, it is classified as an eye irritant or corrosive (EU: R41 "risk of serious damage to eyes", Globally harmonized system, GHS: category 1 "irreversible effects on the eye"). When the induced changes are fully reversible within 21 days of application the compound is classified as eye irritant (EU: R36 "irritating to eyes").

The REACH regulation specifies certain information requirements for substance quantities between ≥ 1 and < 10 tonnes. For substances in this range, if the eye irritation/corrosion potential is not known, additional studies are necessary, it must be observed from in vitro methods only (see REACH, endpoint specific guidance). Thus, there are strong efforts for the entire replacement of the Draize rabbit tests (skin and eye).

Animal experiment (Draize rabbit eye test):

The test substance is placed in the conjunctival sac of one eye of an albino rabbit at a dose of 0.1 ml or 0.1 g. The untreated eye serves as control. After application, the lids are held together for about one second to prevent loss of material. The eyes of the rabbit are then examined after 1, 24, 48 and 72 hours. If immediate corrosion occurs the eyes are rinsed and in case of severe eye lesions and pain the animal should be killed. If no detectable eye lesions occur the test may be terminated after the first 3 days. If lesions are detected the animal must be observed for up to 21 days, during this period the reversibility of the damage is judged. The severity of an eye irritation is judged on the basis of the observed changes in the conjunctiva, eyelids and cornea. In case of an irritant effect or a severe eye irritation, a second test with up to two additional rabbits is obligatory in case of an irritant effect or a severe eye irritation. In case of a severe eye irritation, the animal must be killed. Anticipated painful reactions to irritants.

As a supplement, OECD TG 406 includes an integrated testing strategy (ITS) that provides a weight-of-evidence (WoE) approach for the evaluation of existing data on the eye irritation/corrosion properties of substances and a tiered approach for the generation of relevant data on substances for which additional studies are needed. In this tiered approach validated in vitro and ex vivo methods have to be applied, before the rabbit test according to TG 406 is conducted as last resort.

Four organotypic ex vivo/in vivo assays have been evaluated to identify potential corrosives and severe irritants (OECD TG 406, Annex 1, OECD Test Method 5, relevant Reports). Three of them use ex vivo eye tissue: the rabbit cornea (OECD TG 406, Annex 1, OECD Test Method 5, relevant Reports), the guinea pig cornea (OECD TG 406, Annex 1, OECD Test Method 5, relevant Reports), the rabbit cornea (OECD TG 406, Annex 1, OECD Test Method 5, relevant Reports), and one uses the in vivo properties of embryonated hen's egg chorio-allantoic membranes (HET-CAM). In the EU positive results of these assays are sufficient to classify and label the tested substance as a severe eye irritant/corrosive and further animal testing is avoided (see Manual of Decisions, ECD). Negative results, however, still require subsequent animal testing to discriminate between eye irritants and non-irritants. Several assays to identify non-irritants (and to fully replace the Draize test with a battery of "bottom-up" and "top-down" assays) are under evaluation. Prerequisite for an approval of these methods is a better understanding of the mechanisms that lead to ocular injury and its recovery.

METHOD

Principle:

The chorio-allantoic membrane (CAM) is a translucent vascularised respiratory membrane that surrounds the developing chicken embryo and lines the inner surface of the eggshell beneath the inner shell membrane. It is

The workflow of

method-summary production

urrent state of scientific knowledge

3R forums

Searcher

Antje Dörendahl,
Susanne Skolik

Authors

Barbara Grune,
Daniel Butzke,
Richard Vogel

AnimAlt-ZEBET

Peer reviewer

ZEBET/BfR scientists,
Manfred Liebsch,
Horst Spielmann,
Andreas Luch

triggered by:

- urgency of the issue under consideration (e.g. transgenic animals)
- ongoing requests for a review (by ZEBET experts)
- „white space mapping“ (inventory of animal experiments with no established alternatives yet)

Advisory Services for Public Authorities, Ministries, and Scientists

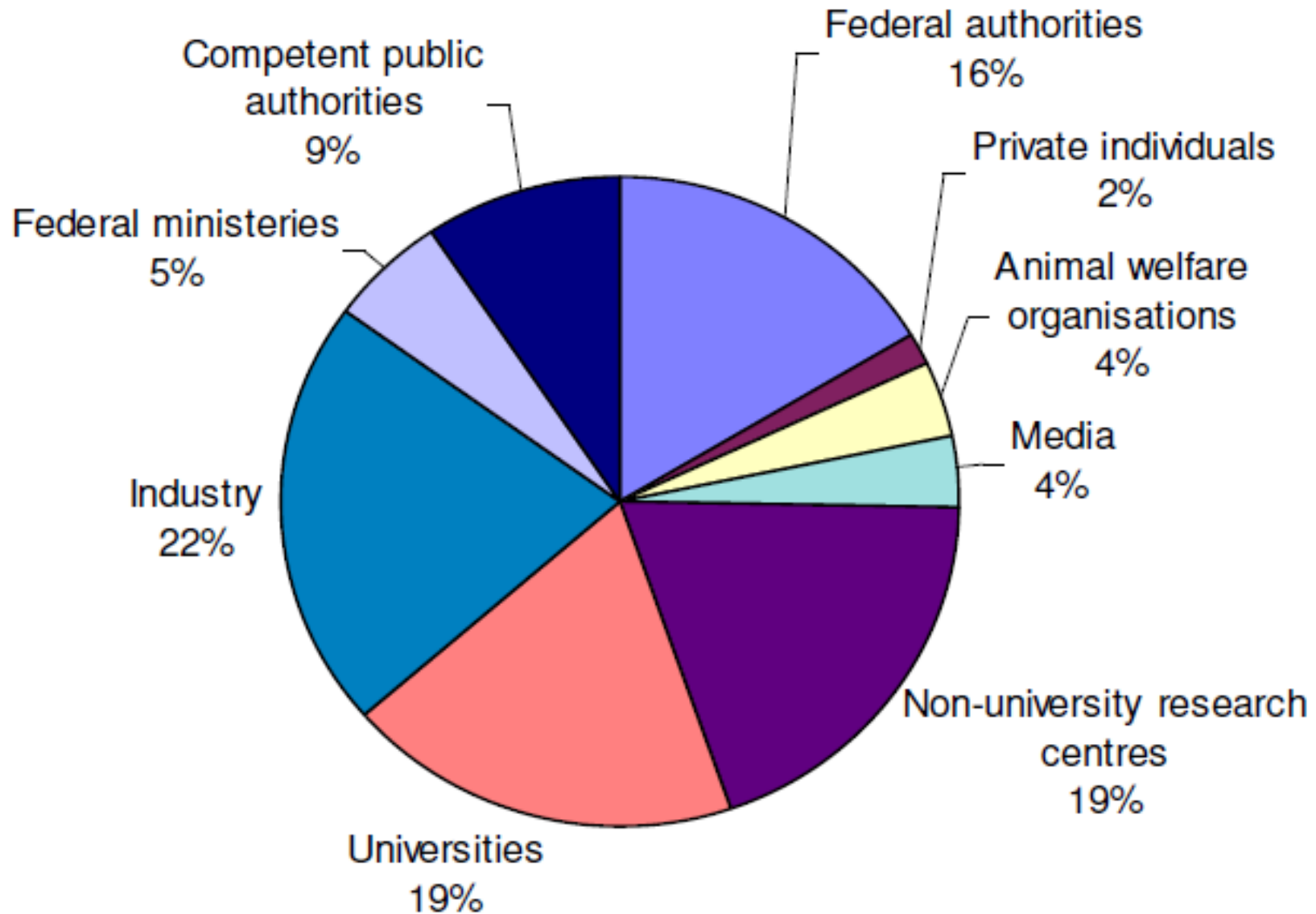
Sara Adler

Federal Institute for Risk Assessment

Centre for Alternative Methods to Animal Experiments -

ZEBET

In 2007 ZEBET responded to 457 inquiries.



Thank you for your attention!

Barbara Grune, Sarah Adler,
Daniel Butzke

Federal Institute for Risk Assessment

Thielallee 88-92 • D-14195 Berlin

Tel. +49 30 - 84 12 - 0 • Fax +49 30 - 84 12 - 47 41

bfr@bfr.bund.de • www.bfr.bund.de