

A woman with long brown hair, wearing a bright green turtleneck sweater, is shown from the chest up. She is holding up her right hand with three fingers extended, symbolizing the 3R principles. The background is a light blue wall with a window grid pattern. A green horizontal bar is overlaid on the image, containing the text.

Reduction – Refinement – Replacement
Cefic engaged in the 3R Principles of Toxicity Testing





Scientific, social, economic and ethical factors are driving the search for alternative methods for safety testing of chemicals that allow the refinement, reduction and replacement of animals for toxicity testing.



The 3Rs

These principles were first defined in 1959 by William Russell and Rex Birch in their book, 'Principles of Humane Experimental Technique'. When employed together they constitute a strategy to both improve animal welfare and minimise overall animal use. The concept is central to guidelines and legislation with such goals.

High Expectations

The search for alternatives has raised high expectations amongst consumers, NGOs and regulators for the replacement of animal testing in the short term.

Science and innovation have made possible the Refinement, Reduction and Replacement of certain testing methodologies that employ animals. Nevertheless, for the immediate foreseeable future, there remain some important aspects of safety assessment that will continue to require the use of experimental animals to ensure effective assessments and the overall safety of our products.

Engagement

Cefic supports the 3R principles and advocates the use of animal testing solely as a last resort, only to be pursued to obtain useful information and in compliance with relevant legislation and the highest animal welfare standards. Cefic engages in collaborations to advance animal alternatives and sponsors research projects through its Long-range Research Initiative (LRI).

Collaborations

- Cefic is a founding member of the European Partnership for Alternative Approaches in Animal Testing (EPAA) which is a joint initiative of seven industry sectors with the European Commission. Together with member companies, Cefic actively participates in the activities engaged by the EPAA Working Groups.

www.epaa.eu.com

- The development of globally harmonised test methods and assessment schemes can significantly reduce testing. This takes place under the umbrella of the Organisation for Economic Co-operation and Development (OECD) Test Guideline programme. Cefic is represented in the various working groups: the Validation Management Group (VMG) non-animal, which deals with establishing in vitro test methods for endocrine disruptors, and the expert group evaluating a new design for reproductive toxicity studies.

www.oecd.org

- LRI is actively involved in the development and acceptance of chemoinformatic methods and (Q)SAR tools. Workshops (ICCA 2002, QSAR 2004, ECB Training Course 2007) were sponsored and projects are being funded to develop data management tools.
- Results of LRI sponsored projects formed the basis of tools such as ToxTree and ToxMatch, initiated by the European Chemicals Bureau (ECB).
- Cefic has participated in the REACH Implementation Projects (RIPs) ensuring that the 3R principles are well integrated (e.g. RIP 3.3 on information requirements).
- Cefic is also promoting the use of Intelligent Testing Strategies (ITS) through the European technology platform for Sustainable Chemistry (SusChem).

Relevance

- Reproductive toxicity testing is required under REACH for substances with a production volume above 100 t per year. This sole test utilises the highest number of animals when compared to other toxicity tests. LRI sponsored a review which forms part of the scientific recommendations prepared by the OECD Expert Group for New design for reproduction toxicity studies in mammals. The review compares the standard 2-generational reproductive toxicity study (TG416) with a modular, enhanced 1-generational protocol.
- LRI is investing in alternative strategies and disseminating the information to encourage their applicability amongst regulators and other stakeholders. With this in mind, collaborative workshops have been organised with partners such as ECVAM, ECETOC, SETAC, CAAT, and others.
- Cefic has engaged on the OECD / ICCA voluntary initiative on High Production Volume (HPV) chemicals which has made extensive use of read-across and other strategies to reduce animal use.

LRI sponsored research on 3Rs



Threshold of Toxicological Concern - TTC REDUCTION

Furthermore, LRI has engaged on research dealing with the evaluation and refinement of the TTC concept. This concept refers to the development of generic human exposure threshold values for groups of chemicals below which no appreciable risk to human health is assumed. The TTC concept addresses the need to screen large numbers of substances present at very low concentrations on a cost-effective manner.

The RepDose database was created that contains repeated dose toxicity studies and helps classify chemicals into combinations of functional groups/categories and toxicological endpoints (i.e. target organs). It features the possibility to store compounds and studies, to pursue chemical structures searches, and a tool for in depth evaluation of reproductive toxicity endpoints (relevant to REACH), amongst others.

LRI has also sponsored a study evaluating an extension of the TTC concept to the assessment of endocrine active substances in aquatic ecosystems.

In Vitro Screening/Tests

REDUCTION

Projects involved in developing strategies to allow the **replacement** of acute and chronic fish toxicity tests via use of fish cell line assays, fish embryo tests and gene expression profiling have been sponsored.

The LRI considers the developmental neurotoxicity testing area to be in critical need of alternatives to current animal testing protocols and guidelines. Therefore, the programme has supported the development of a novel *in vitro* test system to predict developmental neurotoxicity based on human neurospheres using model compounds.

Toxicogenomic tools are being used to accurately identify thresholds of toxicity by determining responses produced by exposures to hormone active agents. If interpreted correctly, these tools can be predictive of sub-chronic or chronic toxicological effects *in vivo*.

Read Across

REDUCTION

LRI has been sponsoring the development of chemoinformatic tools that allow the management of large amounts of data (AMBIT database and OECD (Q)SAR Application Toolbox). These tools permit grouping of chemicals (categorisation) with the goal of filling data gaps (read-across). To populate these data management applications, additional databases are being generated, such as the gold standard database of fish bioconcentration factors (BCF). The BCF database stores information derived from the application of the bioconcentration test (*in vivo* data).

AMBIT database: <http://ambit.acad.bg>

In Vivo Testing

REDUCTION AND REFINEMENT

The use of model organisms to study toxicity testing in order to **reduce** the overall use of animals has been promoted by the LRI. Sponsored projects include those involving the identification of signal transduction pathways with the aim of using these pathways as predictors for mammalian developmental toxicity or detecting molecular mechanisms of carcinogenicity. If validated, they could form the basis of an approach for toxicity testing and to identify human carcinogens, without the need for extensive animal testing.

In order to significantly **reduce** the number of animals used to detect reproductive toxicity of chemicals, the predictive power of the use of one-generational versus multi-generational studies is being analysed.

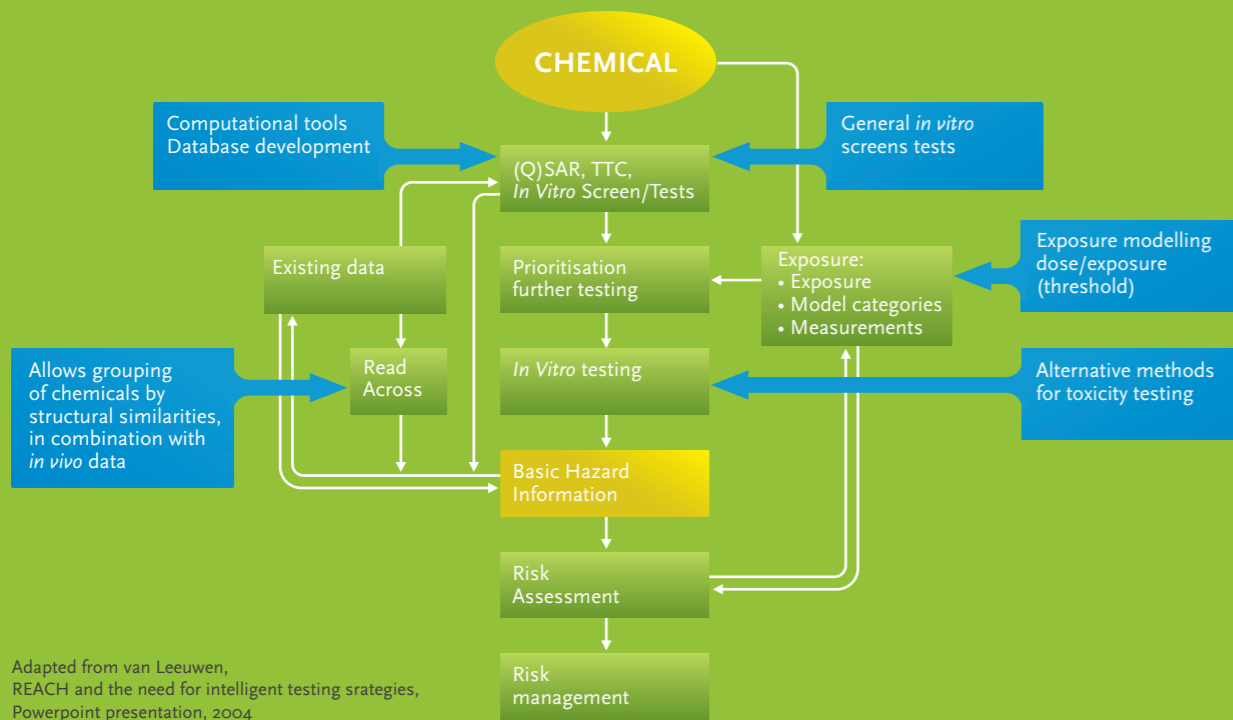
Exposure

REDUCTION

Projects aimed at speeding up the development of physiologically-based pharmacokinetic (PBPK) models, in the field of chemical risk assessment have been pursued. For example, a user interface Model Equation Generator, MeGen, was created that allows the generation and analysis of PBPK models (www.opentox.com/lgen). This modelling tool can be used for rodents as well as other higher species.

To gain a perspective on the relationship of biomonitoring endpoints to actual exposure scenarios, “natural” substances are being used as guides to develop Biological Guidance Values or Biological Equivalents. The LRI has supported the extension of such guides to different substances or classes of material.

Map of LRI sponsored research on the Intelligent Testing flow chart



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Cefic - The European Chemical Industry Council

www.cefic-lri.org

Providing science-based information
to protect the safety of people and
the environment

