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Rethinking the toolbox of toxicology The Centre for Alternative to Animal Testing – Europe (CAAT-EU)

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ABSTRACT: Developing alternative approaches to animal testing and, more in general, updating the instruments toxicologists use to test compounds has become of growing importance. It was 1959 when Russell and Burch set a milestone in the field of toxicology with the publication of their study "The Principles of Humane Experimental Technique", which introduced the concept of the "three Rs", reduction, refinement and replacement, for a more ethical animal experimentation. A long road has been made since then, but the revolution in the contents of the toolbox of toxicology is still pending. In fact, several "traditional" animal based testing procedures developed more than half a century ago are still in use in their unaltered original form. In addition, an ever growing number of evidences pose serious doubts on the real predictive value of animal based testing methods. In Europe, the introduction of the REACH Regulation, the 7th amendment of the Cosmetics Directive posed precise new challenges for toxicologists which cannot be faced with old fashioned methods and approaches. Moreover, in the USA the concept is under development of a Toxicology for the 21st Century, or Tox-21c, focusing on human relevant high throughput methods. Within this context, on March 30th 2010 an agreement was signed between the University of Konstanz (Germany) and the Johns Hopkins Bloomberg School of Public Health in Baltimore (USA) to jointly establish the Center for Alternatives to Animal Testing-Europe (CAAT-EU) in an effort to promote better coordination in toxicity testing. The new centre, modelled after the Bloomberg School's Centre for Alternatives to Animal Testing in Baltimore, will conduct scientific research to find new methods to replace the use of laboratory animals in studies, reduce the number of animals needed for research and refine necessary tests to eliminate the pain and distress of animals in research.

INTRODUCTION

When Russell and Burch published their book "The Principles of Humane Experimental Technique" in 1959, another twenty years had to pass before the *three Rs* concept would be accepted by the scientific community. In addition, method harmonisation, which alone greatly reduces the number of required animal tests, was first implemented only in 1982 with the release of harmonised guidelines for the testing of chemicals by

the Organisation for Economic Cooperation and Development (OECD) and in 1990 for the safety and efficacy testing of drugs by the International Conference for Harmonisation (ICH). The palette of tests usually required by regulatory bodies for safety testing are summarised in Table 1.

Acute systemic toxicity (oral-dermal-inhalation)	Subacute toxicity	Teratology & embryotoxicity
Eye irritation & corrosion	Subchronic toxicity	Reproductive toxicology
Skin irritation & corrosion	Chronic toxicity	Genotoxicity
Skin sensitisation	Metabolism & toxicokinetics	Carcinogenicity
Dermal penetration	Neurotoxicity & immunotoxicity	

Table 1. Animal tests generally required for regulatory purposes.

Alternatives are defined as new methods that refine existing tests by minimizing animal distress, reduce animal usage, or replace whole animal tests. Today, fifty years after the publication of the three Rs concept, reduction, refinement and replacement principles in regulatory safety testing are adopted as a general scientific concept by all governmental and international institutions. Cooperation between industry, academia and regulatory bodies has been fostered by three main drivers: 1. ethical implications of animal testing, 2. high economic impact of "classical" procedures and 3. national/international legislation requirements. Moreover, the growing attention towards international cooperation to best harmonise methods and develop alternatives stimulated the establishment of several laboratories, institutes, joint initiatives, foundations, etc., which have the common task of developing alternative testing methods and to implement the three Rs. A list of three Rs and alternative methods centres can be found in Table 2. The great advances of molecular and cell biology of the last thirty years, paved the road towards the development of novel and straightforward tools for toxicological analysis. Today the bulk of data driving from pure and applied life sciences research along with the development of advanced computing systems are opening the gates to novel *in-vitro* and *in-silico* testing methodologies.

TOX-21C. THE PARADIGM SHIFT IN TOXICOLOGY: FROM ANIMAL TESTING TO HIGH THROUGHPUT TESTING FOCUSED ON HUMANS

An exciting discussion on a novel approach in toxicology, referred to as *Toxicology for the 21st Century* or *Tox-21c*, was initiated 2007 in the USA with the publication of the report of the National Research Council and the National Academy of the Sciences entitled *Toxicity Testing and Assessment in the Twenty-first Century: A Vision and a Strategy*. The driver of *Tox-21c* was the

limited quality and the low throughput of traditional animal testing methods rather than animal welfare considerations. The novel approach wants to exploit high throughput *in-vitro* and *in-silico* technologies with a focus on human biology. Cell culture and tissue culture systems allow the efficient use of human-derived testing materials (cells) employing high throughput technologies to conduct tests and to analyze specimens. Systems biology is a powerful approach to make evaluations by joining computational models and laboratory data to describe and understand the functioning of biological systems. Finally, the use of bioinformatics for the analysis of high amounts of information will allow the development of powerful predictive tools.

A general scheme of this novel approach is provided in Figure 1. At the centre of toxicity testing the assessment of toxic pathways will be complemented by targeted animal testing trials designed to clarify and refine information from toxicity pathway tests, since it is not possible to test in cell cultures the complex network of interactions, which e.g. lead to the formation of breakdown products of tested compounds. Dose-response and extrapolation modelling will allow extrapolating from cell based tests data for whole human systems and, more specifically, it will estimate environmental exposures that would lead to significant perturbations of the toxicity pathways observed in the cellular tests. Population-based and human exposure data will represent another aspect of evaluation. Finally, also risk context evaluation is of huge importance since it is often allowing reducing the need for lengthy and costly dose-response modelling.

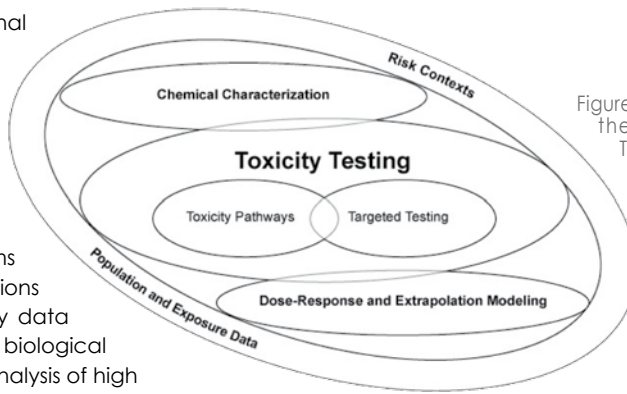


Figure 1. Toxicity testing in the vision of Toxicity Testing and Assessment in the Twenty-first Century: A Vision and a Strategy (from the report).

THE JOHNS HOPKINS CENTER FOR ALTERNATIVES TO ANIMAL TESTING (CAAT)

The Johns Hopkins Centre for Alternatives to Animal Testing (CAAT), directed by Thomas Hartung, Doerenkamp-Zbinden Professor and Endowed Chair for Evidence-based Toxicology, is located in the Johns Hopkins University in Baltimore, Maryland, USA. The Centre was founded in 1981 with a grant of the Cosmetic, Toiletry and Fragrance Association (CTFA) with the aim to develop basic scientific knowledge necessary to create innovative non-whole animal methods for evaluating the safety and efficacy of commercial and therapeutic products. CAAT was founded in a period when the public opinion was heavily criticising scientific community and toxicologists for the use of animal testing. Moreover, some toxicologist had expressed their reservations about testing procedures not sufficiently taking into account progresses in scientific research. The idea

to employ alternatives, at least to some animal testing, was developing. Within this context, the idea underpinning the CAAT was to explore the possibility of developing *in-vitro* or other alternatives (e.g. *ex-vivo*) to the use of animals. It is worth noticing that at that time no one could predict that safety evaluation would have been possible without the use of animals. In more than 25 years of activity the CAAT was able to collect several achievements. Besides the top level science, which is done within the centre and the development of several *in vitro* testing methods currently in use, the CAAT has gained a pivotal role in knowledge dissemination and in bringing together academic and industrial scientists, policy makers and animal welfare organisations. The vision of CAAT is to be a leading force in the development and use of reduction, refinement, and replacement alternatives in research,

Institution	Name	Country
ANZCCART	Australian and New Zealand Council for the Care of Animals in Research and Teaching	Australia New Zealand
APHIS	Animal and Plant Health Inspection Service (USDA)	U.S.A.
ARDF	Alternatives Research & Development Foundation	U.S.A.
AWIC	Animal Welfare Information Centre	U.S.A.
CAAT	The Johns Hopkins Centre for Alternatives to Animal Testing	U.S.A.
CAAT - EU	The Centre for Alternatives to Animal Testing - Europe	Europe
CCAC	Canadian Council on Animal Care	Canada
CARDAM	Centre for Advanced Research & Development on Alternative Methods	Belgium
CAAE	Centro de Alternativas al uso de Animales en la Enseñaza	Mexico
ECOPA	European Consensus-Platform for Alternatives	Europe
ECVAM	European Centre for the Validation of Alternative Methods	European Commission
EPAA	European Partnership for Alternative Approaches to Animal Testing	Europe
EURCA	European Resource Centre for Alternatives in Higher Education	Europe
FRAME	Fund for the Replacement of Animals in Medical Experiments	U.K.
GTEMA	Spanish Group on Alternative Methods	Spain
HSUS	Humane Society of the United States	U.S.A.
I-CARE	International Centre for Alternatives in Research and Education	India
ICCVAM/NICEATM	Interagency Coordinating Committee for the Validations of Alternative Methods / NTP Interagency Centre for the Evaluation of Alternative Toxicological Methods	U.S.A.
ILAR	Institute for Laboratory Animal Research	U.S.A.
Institute for the 3Rs	The Institute for the 3Rs	Korea
JaCVAM	Japanese Centre for the Validation of Alternative Methods	Japan
KSAAE	Korean Society of Alternatives to Animal Experiments	Korea
NC3Rs	National Centre for the Replacement, Refinement and Reduction of Animals in Research	U.K.
NCA	Netherlands Centre for Alternatives to Animal Use	Netherlands
Norecopa	Norwegian Reference Centre for Laboratory Animal Science and Alternatives/Norwegian National Platform for Alternatives	Norway
Prince Laurent Foundation	Fondation Prince Laurent pour le bien-être des animaux domestiques et sauvages	Belgium
REMA	Spanish National Platform on Alternatives	Spain
RSPCA	Royal Society for the Prevention of Cruelty to Animals	U.K.
SCAW	Scientists Centre for Animal Welfare	U.S.A.
UCCAA	University of California Centre for Animal Alternatives	U.S.A.
ZEBET	Centre for Documentation and Evaluation of Alternative Methods to Animal Experiments	Germany

Table 2. Three-Rs and alternative testing centres or initiatives worldwide.

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testing, and education to protect and enhance the health of the public. The stated mission of CAAT is to:

- Promote and support research in the development of *in vitro* and other alternative techniques.
- Serve as a forum to foster discussion among diverse groups leading to creative approaches to facilitate acceptance and implementation of alternatives.
- Provide reliable information on the science, philosophy, and public policy of alternatives to academia, government, industry and the general public.
- Educate and train in the application of alternatives.

THE CENTER FOR ALTERNATIVES TO ANIMAL TESTING - EUROPE (CAAT)

The University of Konstanz in Germany and the Johns Hopkins Bloomberg School of Public Health jointly established the Centre for Alternatives to Animal Testing–Europe in an effort to promote better transoceanic coordination in toxicity testing. The Centre was inaugurated on March 30th 2010 with an inaugural Symposium Chaired by Thomas Hartung, Director of the CAAT in Baltimore with speeches held by Ulrich Ruediger (Rector of the University of Konstanz), Michael Klag (Dean of Johns Hopkins Bloomberg School of Public Health), Alan Golberg (Professor of Toxicology at the Johns Hopkins University), Michael Leist (Director of the CAAT-EU, University of Konstanz), Gerd Gantefoer (Chair of Cluster Physics at the University of Konstanz and Professor at the Johns Hopkins University), Michael Balls (Professor Emeritus at the University of Nottingham and Editor of ATLA), Horst Spielmann (retired Director at ZEBET and Professor at the Freie Universitaet Berlin). Moreover a series of speeches were held by experts of the sponsoring organizations, among others the EuroGroup for Animals, an umbrella organization made up of 30 and more animal protection organizations and the ECOPA, a consensus platform for alternative methods made up of representatives of academia, industry, animal protection organizations and governments.

The take-home message outlined by Thomas Hartung and Colleagues during the symposium was clear. The current traditional safety evaluation systems should be considered an expensive and lengthy patchwork developed to fill regulatory needs and leaving huge gaps open here and there. Pushing forward toxicology to gain better predictivity, more adherence with the human "model", high throughput and to achieve the three-Rs, does not mean adding new methods to the toolbox of toxicology but, instead, means replacing outdated methods with

straightforward ones. In addition to that, validation procedures for regulatory acceptance of testing procedures should be deeply rethought. Validation schemes currently in use are too rigid, costly and lengthy if confronted with the actual needs of flexibility and of fast method development. Current procedures risk freezing the palette of methods which gained regulatory acceptance. In fact, at least theoretically, every variation of a protocol requires a *de novo* validation. This is associated to a high cost in terms of money and time. More flexible and faster validation schemes should be implemented like modular validation or retrospective validation, which allow to revalidate only a component of an experimental procedure and to employ the bulk of already existing data.

The CAAT – EU, directed by Marcel Leist, Doerenkamp-Zbinden Chair for Alternative *in-vitro* methods, University of Konstanz, is modelled on the basis of the CAAT in Baltimore and will conduct scientific research to develop methods aimed to replace the use of laboratory animals, to reduce the needed number of animals and to refine those still necessary tests to eliminate the pain and distress of animals. The CAAT - EU was set up to create a transatlantic bridge for the paradigm shift in toxicology towards the Tox-21c concept. Such a bridge is of great importance if we consider the differences in approach which exist between Europe and the U.S.A. In Europe REACH,

3M	ExxonMobil Biomedical Science, Inc.
Abbott Laboratories	GlaxoSmithKline
Alberto-Culver Company	Humane Society of the US
Allergan	Johnson & Johnson
Alternatives Research and Development Foundation	Kimberly-Clark
American Chemical Council	L'Oréal (CAAT-EU)
Avon	Lucille Ellis Simon Foundation
BASF (CAAT-EU)	Materials (RIFM)
Beiersdorf (CAAT-EU)	Merck
Bernice Barbour Foundation Inc.	PepsiCo
Bristol-Myers Squibb	Pfizer
Charles River Foundation	Procter & Gamble Company
Clorox Company	Research Institute for Fragrance
Dial Corporation	Shell Oil Company
Doerenkamp-Zbinden Foundation (CAAT-US and CAAT-EU)	The Coca-Cola Company

Table 3. Companies and foundations supporting CAAT and/or CAAT-EU.



Thomas Hartung, Director of CAAT, Dr. Michael Leist, Director of CAAT-EU and Ulrich Ruediger, Rector of the University of Konstanz (from left to right).

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the 7th Amendment of the Cosmetics Directive and the new Cosmetic Regulation set precise requirements for the testing of synthetic chemicals and the reduction of animal testing procedures, while in the USA the interest is pushed towards the development of high throughput human relevant techniques for regulatory safety control purposes. Harmonization of procedures is therefore important both to best implement analytical systems and to avoid useless duplication of safety tests for regulatory purposes. To this regard, a *Transatlantic Think Tank for Toxicology* (t⁴) is under development joining CAAT, CAAT EU and partners in Utrecht, NL, to enable conceptual work to be developed for the paradigm shift in toxicology without pressures of regulators or of proposing regulations.

A major task of the CAAT-EU is to promote a better communication between the various involved subjects of academia, industry, animal welfare and governmental sectors. CAAT and CAAT-EU are supported and funded by several companies and foundations listed in Table 3.

Declared goals of CAAT-EU are:

- Establish a CAAT EU faculty and advisory board composed of sponsor representatives and prominent academics from all over Europe.
- Establish a competence base of European experts available for project work.
- Participate in the Transatlantic Think Tank for Toxicology (t⁴) devoted to conceptual work for the paradigm shift in toxicology.
- Coordinate a series of information days on relevant developments from the US in Europe, a reciprocal of the program already established by CAAT in the US.
- Organization of workshops on relevant topics, regarding toxicological, biomedical, education related and ethical issues.
- Set up transatlantic consortia for international research projects on alternative methods.
- Support ALTEX as the official journal of CAAT, EUSAAT, and t⁴.
- Develop strategic projects with sponsors to promote humane science and new toxicology.

CONCLUSIONS

Today's toxicology needs a deep rethinking in order to modernize procedures and eliminate outdated methods. Moreover, the developments of modern molecular and cell biology, of computing applied to biological systems, and the growing number of scientific data, allows the shift towards a toxicology basing more on *in-vitro* and *in-silico* analysis rather than on *in-vivo* tests on animals. The CAAT-EU is starting its activity with the precise goal to pose a transatlantic bridge to join European and North American efforts to develop new testing methods. Moreover, the CAAT-EU wants also to harmonize and coordinate efforts in the frame of the two visions of toxicology developing in Europe and the USA. In Europe the research on alternative methods in safety analysis is driven by regulatory needs deriving from regulations like REACH or the Cosmetics Regulation, while in the USA the idea was developed to focus more on high throughput high quality analysis having the human model at its centre rather than animal models within the context of the Tox-21c discussion.

REFERENCES AND NOTES

Useful readings and links on CAAT and the three Rs:

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- Website of the Universität Konstanz <http://www.uni-konstanz.de>

For more bibliography please check out the website of the Johns Hopkins Center for Alternatives to Animal Testing.

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