A Chair on Alternatives?*

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Summary
An overview is given on the legal framework in Europe for the use of experimental animals set by EU (European Union) Directive 86/609/EEC and on the activities of EU member states to implement this directive in the field of regulatory testing in animals. The significant decrease in the number of experimental animals in Germany during the past decade is described with particular reference to the recent increase that is due to transgenic animal models. From the regulatory and the animal welfare perspective the international harmonisation of test guidelines and the mutual acceptance of data are the way forward for chemical safety testing. The recent White Paper of the EU Commission for the future chemicals policy calls for an immediate increase in the number of validated in vitro toxicity tests to be accepted for regulatory purposes in the EU. In addition, deficits in the proper education of scientists in Germany in conducting animal experiments and implementing the 3-Rs concept of Russel and Burch are described. Therefore, it is quite urgent to establish new chairs on animals and alternatives at universities in Europe. They should focus on both education of young students of the biomedical sciences in the humane use of laboratory animals according to the 3-Rs concept and on developing new toxicity tests to be validated for regulatory purposes under the new EU chemicals policy by the established validation centres in Europe.

Zusammenfassung: Ein Lehrstuhl für Alternativen?
Es wird eine Übersicht über die gesetzlichen Rahmenbedingungen für die Durchführung von Tierversuchen in den Mitgliedsstaaten der EU im Rahmen der EU Richtlinie 86/609/EWG gegeben sowie über die Aktivitäten der EU Mitgliedsstaaten zur Reduzierung von Tierversuchen, die für behördliche Zwecke vorgeschrieben sind. Es wird besonders die starke Abnahme der Tierversuche in der EU und in Deutschland im Zeitraum des Jahrzehnts von 1989-1999 betont und auch der unerwartete Anstieg der Tierversuchszahlen in den EU Mitgliedsstaaten in den letzten drei Jahren, der auf die zunehmende Verwendung von transgenen Tiermodellen zurückzuführen ist. Aus Sicht der Behörden in den EU-Mitgliedsstaaten ist eine weitere Verminderung der behördlich vorgeschriebenen, sicherheitstoxikologischen Tierversuche vor allem durch die internationale Harmonisierung zu erzielen, wie z.B. auf Ebene der OECD, und durch die gegenseitige, internationale Anerkennung der Tierversuchsdaten. Das im Jahr 2001 von der EU Kommission vorgelegte „Weißbuch für eine neue Chemikalienpolitik" sieht den verstärkten Einsatz tierversuchsfreier Prüfmethoden bei der sicherheitstoxikologischen Bewertung von Gesundheits- und Umweltrisiken chemischer Stoffe vor.
Es werden außerdem die derzeit in Deutschland bestehenden Defizite bei der Ausbildung junger Wissenschaftler in der Durchführung von Tierversuchen beschrieben, für die es derzeit keine verbindlichen Vorschriften gibt und in deren Rahmen das 3-R-Prinzip von Russel und Burch vielfach nicht berücksichtigt wird. Es erscheint deshalb vordringlich, neue Lehrstühle für Alternativmethoden zu Tierversuchen an Hochschulen in Deutschland und anderen europäischen Ländern zu etablieren, die einerseits junge Wissenschaftler aus allen Gebieten der experimentellen Biomedizin mit dem humanen Umgang mit Versuchstieren entsprechend dem 3-R-Prinzip von Russel und Burch vertraut machen. Sie sollten außerdem die Entwicklung neuer tierversuchsfreier Toxizitätstests vorantreiben, die es im Rahmen einer zukünftigen Chemikalienpolitik der EU gestatten, die toxikogische Risikobewertung unter Gesundheits- und Umweltschutzaspekten ohne Daten aus Tierversuchen durchzuführen.

Keywords: university chairs, animal welfare, alternative tests, Doerenkamp-Zbinden-Foundation, 3-Rs concept, reduction, refinement, replacement, education, toxicity tests, regulatory testing, ECVAM, European Commission

1 Introduction

In 1959 William Russel and Rex Burch published the book "Principles of humane experimental technique", in which they suggested the "3-Rs concept" (refinement, reduction and replacement) for the humane treatment of experimental animals (Russel and Burch, 1959). 40 years later the "3-Rs concept", which had not been appreciated by the scientific community for about 20 years, has become the generally accepted scientific concept of government institutions serving the development of alternatives to regulatory safety testing in animals, e.g. ECVAM (European Centre for the Validation of Alternative Methods in Ispra, Italy) and ZEBET (German National Centre for the Documentation and Evaluation of Alternatives to Animal Experiments in Berlin) in Europe and ICCVAM (Interagency Co-ordinating Committee for the Validation of Alternative Methods at the NIEHS in Research Triangle Park) in the USA. However, at the university level in Germany chairs promoting research according to the 3-Rs concept have not been established. In contrast, at the respected Johns Hopkins University in Baltimore, CAAT, the Center for Alternatives to Animal Testing, has actively promoted and funded research according to the 3-Rs for the past 20 years. Taking into account the high priority that EU member states as well as the EU Commission (EC) are giving animal welfare and the reduction of animal numbers used for scientific purposes, European universities should be encouraged to follow the example of their American colleagues and establish chairs on alternatives. Such chairs should focus both on research to develop new test methods that can be validated at an international level and on educating young scientists on applying the 3-Rs concept in their scientific careers.

2 Legal framework in Europe for the use of experimental animals

According to article 7.2 of EU Directive 86/609/EEC on the use of experimental animals, "an experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practicably available" (EC 1986). To promote the implementation of the EU Directive 86/609/EEC on the use of experimental animals the European Commission and several member states have established centres for the validation of alternative methods, e.g. ZEBET in 1989 and ECVAM in 1992. Reducing regulatory testing in animals is ZEBET's and ECVAM's main task. In the USA, ICCVAM is serving a similar mission.

Since ZEBET was established in 1989, the annual numbers of experimental animals in Germany have decreased from 2.7 Mio in 1989 to 1.6 Mio in the year 1999. A closer analysis shows that the decrease is predominantly due to a reduction in animal numbers used for the development of drugs, which decreased by 50% from 1.4 Mio in 1989 to 0.6 Mio in 1999. This dramatic development is due to a general change of the methodology used in drug development from animal models to molecular biology and genetics, including cell and tissue culture models. However, due to the increase in the use of transgenic animals or GMOs (genetically modified organisms), experimental animal numbers have increased significantly in Europe during the past 2 years.

3 Reducing animal numbers in regulatory testing by international harmonisation of test guidelines

For the past 30-40 years toxicity testing has been developed empirically in many laboratories around the world. Table 1 gives a summary of toxicity tests which are required for regulatory purposes today. For economic reasons companies that have to provide testing data to regulators have used flexibility and common sense to convince national and international regulatory agencies that harmonisation of test guidelines is the only way forward in a world-wide economy. In 1982 the OECD was the first international organisation that agreed on harmonised guidelines for the testing of chemicals (OECD 1982). A similar approach was decided by the International Conference on Harmonisation (ICH) in 1990 for safety and efficacy testing of drugs (D'Arcy and Harron,

Tab. 1: Toxicity testing of chemicals: the current animal methods

- acute systemic toxicity (oral, dermal, inhalation)
- eye irritation & corrosion
- skin irritation & corrosion
- skin sensitisation
- dermal penetration
- subacute toxicity
- subchronic toxicity
- chronic toxicity
- metabolism & toxicokinetics (ADME)
- neurotoxicity & immunotoxicity
- teratology & embryotoxicity
- reproductive toxicology
- genotoxicity
- carcinogenicity

The table summarises the current animal safety tests which generally have to be conduced for regulatory purposes.

ADME = absorption, distribution, metabolism and excretion.

Tab. 2: International harmonisation of guidelines for toxicity testing in animals

- Industrial chemicals, food additives and contaminants, pesticides, cosmetics etc:
  OECD-Guidelines for the Testing of Chemicals, EU (SCF), FAO/WHO (JACFA)
- Drugs and medical devices:
  International Conferences on Harmonisation (ICH)
- Safety and efficacy of hormones and biological:
  Pharmacopoeias (European Pharmacopoeia Commission, US Pharmacopoeia)
- Vaccines and other immunologicals:
  WHO recommendations, European Pharmacopoeia Commission

1995). Again, harmonisation of test guidelines led to significant reduction of testing in animals.

Table 2 summarises the most important areas that require safety testing in animals and in which the test guidelines have been harmonised at the international level. Table 2 shows that in addition to drugs, industrial chemicals, food additives and pesticides, international test guidelines have also been harmonised for hormones and biologicals by the pharmacopoeias and for vaccines by the WHO. So far, the harmonisation of international test guidelines for toxicity and safety testing has been the most successful approach to reducing animal testing for regulatory purposes.

In the EU official test methods for chemicals are published in Annex V to Directive 67/548/EEC (EC 1976) on the classification, packaging and labelling of dangerous substances. In the year 2000, two experimentally validated *in vitro* toxicity tests were added to part B of Annex V for the first time, the 3T3 NRU (Neutral Red Uptake) *in vitro* phototoxicity test, which was developed by ZEBET, and an *in vitro* skin corrosivity test (EC 2000). In the EU, methods of part B of Annex V are used for the determination of hazardous properties of chemicals to human health as described in Figure 1. These methods are used for new and existing chemical substances, food additives, cosmetic ingredients, plant protection products, biocides etc. The Annex V methods are continuously harmonised with other relevant international test programs (e.g. OECD).

The use of test methods for risk management in Europe

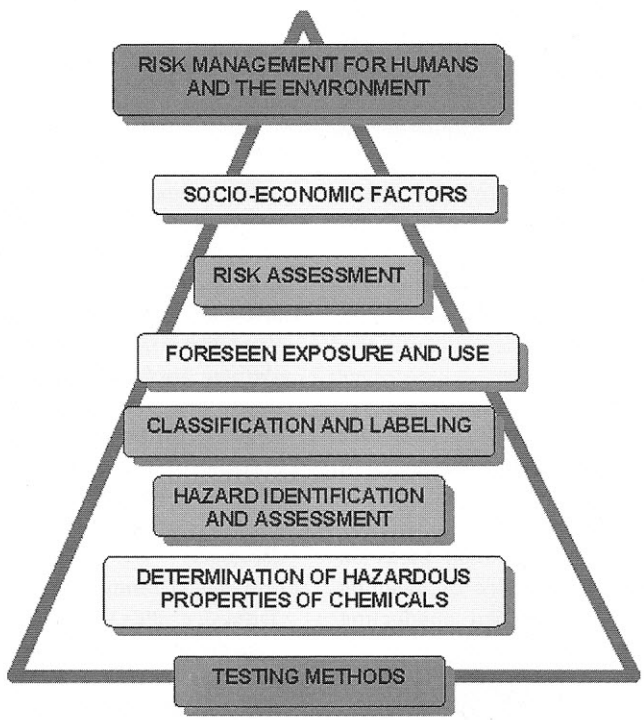

Fig. 1: The use of test methods for risk management in Europe
The graph describes the use of test methods published in Annex V of EU Directive 67/548/EEC in the hazard and risk assessment process and also for risk management for humans and the environment.

4 White Paper of the EU Commission: a new chemicals policy that takes into account exposure and data from *in vitro* toxicity tests

The current chemicals regulation in EU member states provides sufficient data on new chemicals but almost none on the about 100,000 existing chemicals (EU 2001a). To improve the situation, the EU Commission has proposed the strategy for a new chemicals policy to regulate new and existing chemicals in an identical manner in a recent White Paper published in 2001. The Commission suggests to use *in vitro* methods to provide rapid information on hazardous health and environmental properties of chemicals. The fundamental change in the testing strategy, which relies on *in vitro* methods rather than testing in animals is most welcome, not only from the scientific and economic point of view, but also from the perspective of the protection of animals and the environment. A calculation of the costs and time for testing all existing chemicals in animal experiments shows that this task is unrealistic from a financial point of view and also when taking into account the time required for testing. During the past year, an expert working group of the EU validation centre ECVAM has developed a concept that will allow testing of the majority of existing chemicals by applying only non-animal tests. Since the

new testing strategy will be cheaper and faster, it will be more acceptable from both the financial and the ethical point of view. Therefore, representatives of the EU chemical manufacturers association CEFIC (European Chemical Industry Council, Brussels) and of the Euro Group of animal welfare organisations have welcomed the new EU chemicals policy. Finally, establishing the proposed new *in vitro* tests in the safety testing strategy will require considerable funding for the development and validation of new non-animal safety tests.

5 Educating young scientists in the ethical use of animals according to the 3-Rs concept

According to EU Directive 86/609/EEC for the use of experimental animals scientists have to implement ethical principles according to the 3-Rs concept, not only when conducting experiments but also when using animals in education and training courses. Therefore, in several EU member countries educational courses in laboratory animal sciences including the 3-Rs concept are mandatory for students and young scientists in the life sciences, e.g. in the Netherlands. In many universities in Germany, however, for legal reasons, in particular due to the constitutional rights of "freedom of research and teaching" (Freiheit von Forschung und Lehre), the 3-Rs concept has so far not been implemented into education and training courses. There are, however, a few exceptions, e.g. in Berlin at the Humboldt Universität in collaboration with ZEBET, a course entitled "Versuchstierkunde, Tierversuche und Alternativmethoden", which is certified by the German Society of Laboratory Animal Sciences, is mandatory for students and young scientists who conduct animal experiments for scientific purposes.

Meanwhile, at the international level, in the year 2000 at the 52nd World Medical Association (WMA) General Assembly, the WMA amended § 12 of the "Ethical Principles for Medical Research Involving Human Subjects" which reads now "Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected" (WMA 2000). Moreover, in the same year, the European Science Foundation (ESF), a non-governmental association of leading national science funding agencies in 23 European countries, accepted a policy briefing entitled "Use of animals in research", which not only strongly endorses the principle of the "Three Rs" but also recommends that "investigators and other personnel involved in the design and performance of animal-based experiments should be adequately educated." (ESF 2000). The Deutsche Forschungsgemeinschaft (DFG), which is a member of the EST, therefore has the obligation to help implement this recommendation into the curriculum of students and young scientists at universities in Germany, since the DFG funds most of the research at universities in Germany.

However, since such courses are expensive, they have so far not been given the priority that they must be given for both legal and ethical reasons for financial reasons. Thus, establishing chairs for alternatives at universities in Germany would significantly improve the situation and add momentum to implementing the 3-Rs concept at the university level in Germany.

6 Chairs on alternatives should focus on education and research

As outlined above, chairs for alternatives to animal experiments at universities in Germany should cover the needs of implementing the 3-Rs concept into education and in research.

Education activities should focus on the most humane practice of handling experimental animals and conducting experiments. Therefore, the following topics should be covered: knowledge of the biology of the most common laboratory animal species; housing and handling of laboratory animals; searching for alternative methods in the scientific literature, including databases on the internet; legal and ethical aspects of animal experimentation in EU member states; alternatives to animal experiments according to the 3-Rs concept, and finally the best and most humane practice to conduct animal experiments. As long as alternatives do not exist to all animal experiments, such courses will have to include experimental practice on laboratory animals.

Chairs on alternatives should focus their research activities on the development of alternatives that will support the EU White Paper on the future chemicals policy in Europe. Therefore, research is needed in applied rather than in the basic sciences in order to develop *in vitro* alternative methods for the safety assessment of chemical substances irrespective of their use in industry or by the consumer, e.g. drugs, cosmetics and pesticides. The most promising methods will then be experimentally validated by the EU via ECVAM. Currently funding of this particular area has a high priority in the 6th Framework Program (FP) of the DG Research of the EU Commission, since specific funding has been allocated for this particular purpose during the years 2002-2006 (EC 2001b).

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