Symposiumsbericht

Symposium „ZEBET's 10 Year Anniversary"

21st-22nd June 1999 at the BgVV in Berlin

Das Symposium zum 10jährigen Jubiläum von ZEBET war ausserordentlich gut besucht. Etwa 150 Gäste aus befreundeten Institutionen des In- und Auslandes, aus Tierschutzkreisen, Universitäten und Industrie machten ihre Aufwartung. Leider war es dem für Tierschutz zuständigen Bundesministerium für Landwirtschaft und Forsten nicht möglich, eine Vertreterin oder einen Vertreter zu schicken.

Das wissenschaftliche Programm war anspruchsvoll, im folgenden werden die Aufsätze bzw. Kurzfassungen der jeweiligen Beiträge präsentiert. Einige der Referate wurden als Hauptartikel bzw. Kurzmitteilungen bei ALTEX eingereicht und entsprechend dem Redaktionsstatut einem Review-Verfahren unterzogen.

Den Höhepunkt des ersten Tages bildete zweifelsfrei die Laudatio der Bundesministerin für Gesundheit, Andrea Fischer, und die Verleihung des Forschungspreises für Alternativmethoden 1998.

Frau Fischer ging auf die Geschichte von ZEBET ein und sprach an, dass dieser Einrichtung anfangs eine gehörige Portion Skepsis, wenn nicht gar offenes Misstrauen entgegengebracht wurde. Doch Qualität setzt sich durch, und dies drückt sich letztlich nicht nur in den Forschungspreisen aus, die ZEBET bisher erhalten hat, sondern auch in der Kooperation mit den entsprechenden Agenturen und Behörden des Auslandes und von supranationalen Organisationen.

Die Ministerin nahm die Gelegenheit wahr, um auch ein Statement zur Tierschutzpolitik der Bundesregierung abzugeben. Sie ging dabei auf die geplante Verankerung des Tierschutzes im Grundgesetz ein und sprach die Überzeugung aus, dass damit dem Tierschutz endlich aus der Rolle des ewigen Zweiten hinter der Forschungsfreiheit geholfen werden könne.

Mit bewegten Worten überreichte Frau Fischer schliesslich den Forschungspreis des Ministeriums an Frau Siegel-Axel aus Tübingen für ihre Leistungen auf dem Gebiet der Entwicklung von Alternativmethoden zur Erforschung der Atherosklerose.

Die Preisträgerin bedankte sich mit einem Referat, in dem sie ihre Forschungsergebnisse der letzten Jahre darstellte, aber auch sehr realistische Abschätzungen über den zukünftigen Einsatz ihres Kokulturmodells aus menschlichen Gefässzellen gab (s. Hauptartikel in diesem Heft).

Die Laudatio von Frau Minister Fischer folgt in vollem Wortlaut.

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Andrea Fischer

Federal Minister of Health, D-Bonn:

10th anniversary of ZEBET and the Federal Ministry for Health Research award 1998 to promote methodological work aimed at reducing and replacing of animal experiments

Prof. Arnold *,
Prof. Spielmann **,
Distinguished Guests,

I am happy to be able to welcome you here in Berlin, on behalf of the Federal Government, to the symposium celebrating the 10th Anniversary of the founding of the Centre for Documentation and Evaluation of Alternatives to Animal Testing. My colleague who is responsible within the Federal Government for animal protection and welfare, the Federal Minister of Agriculture, has asked me to extend his warmest greetings to you as well.

I am particularly pleased that it has been possible to hold this symposium before the German EU Presidency comes to a close as it gives us the possibility to illustrate even more clearly the significance of animal protection and the fact that it extends beyond the national framework. A very special welcome, therefore, to all of our foreign guests as well.

ZEBET

The Centre for Documentation and Evaluation of Alternatives to Animal Testing, in German ZEBET for short, managed in the short space of ten years to develop into an institution of national and international renown. As it's so often the case with something good, a number of persons have made a decisive contribution to this success story. I would like to thank professor Spielmann and his co-workers as the representatives of all of those persons, who have made this success possible.

It all began in November 1986 with the adoption of the Council Directive concerning the protection of animals used for experimental and scientific purposes. This directive called upon the Commission and the Member States of the European Community to develop and evaluate alternative methods to animal testing as well as to promote research in this field. It was in the context of the adaptation of German legislation on animal protection to this Directive – pursuant to which authorisations for animal experiments may no longer be

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granted if suitable alternative or supplementary methods are available - that ZEBET was founded in 1989. For all concerned, it was obvious that, without a central institution to compile, evaluate and ensure, the recognition of alternative and supplementary methods to animal experiments, the new provisions and regulations would come to naught. The founding of ZEBET was a means of satisfying the rightful demands of animal protection organisations, informed members of the public and of all the parliamentary groups in the German Bundestag.

However, it cannot be said that ZEBET was greeted only with expressions of goodwill and support in those days. At that time, many scientists expressed scepticism as to whether it would be possible to develop alternatives, so-to-speak, from nothing. At the same time, there were fears that ZEBET's activities would lead to increased attempts to exercise control as well as to a greater bureaucratisation of research at the universities and in industry.

These fears must have been allayed at a very early stage, I would think, since nowadays animal protection activists, industry and science collaborate exceedingly well with ZEBET.

The protection of animals used for experimental purposes is an area of policy which is influenced to a decisive degree by the Council of Europe, the European Union and the OECD. As a result, ZEBET was also entrusted with the task of bringing its expert knowledge to bear on the international regulations. As early as 1991, ZEBET was entrusted with the co-ordination of European validation projects as a result of the competence conceded to it within Europe. Close co-operation exists with the European Centre for the Validation of Alternative Methods (ECVAM), the EU agency which co-ordinates national activities within the European Community and which is meant to strive for recognition of the new methods outside of the European Community.

In the meantime, ECVAM, in collaboration with ZEBET, has concluded a series of successful validation studies. From 1994 to 1995 for example, all of the information available on the 60 substances which are authorised for use under the graduated plan for the classification of corrosive characteristics currently being adopted by the OECD were evaluated. The study revealed that testing with rabbits is no longer necessary for this purpose.

Absolutely indispensable for the worldwide acceptance of the alternative methods validated in Europe is co-operation with America's Interagency Co-ordinating Committee for the Validation of Alternative Methods (ICCVAM) which represents a total of 14 American federal authorities. One patent indication of how well this co-operation works is the attendance at today's event.

Another task which falls to ZEBET is the initiation of research and validation projects wherever promising signs of potential development and evaluation of alternative methods to animal experiments become known. Since 1990, ZEBET has been promoting corresponding research projects. This year alone, it will be doing so to the tune of 680,000 Deutsche Marks. In addition to this, the Centre also carries out its own research projects. Evidence of ZEBET's success in this area is the two renowned international research prizes it was awarded in 1997 for the successful development of a test and for the completion of an international validation study.

This year too, prizes were received. Professor Spielmann and Professor Balls, the Head of the EU Validation Centre (ECVAM) shared the prize of the Swiss "Fondation Egon Naef": for the development and validation of an *in vitro* phototoxicity test. The test developed by ZEBET serves as the basis for the drafting of a test guideline, which the European Commission submitted to the OECD in September 1998. Experience shows that the OECD's voting procedure takes two years so that a decision can be expected, at the earliest, in the year 2000. If the OECD includes this guideline in its official collection, it will be the first guideline to achieve this status and as such will gain world-wide acceptance.

An important instrument for the dissemination of up-to-date knowledge about alternative and supplementary methods is databases. Through DIMDI, one of the Federal Ministry for Health's subordinate institutes, the ZEBET database is scheduled to be made available online in English before the end of this year. This database provides information on alternative and supplementary methods and the uses to which they can be put. The data in question can be used, for example, by scientists, by persons responsible for animal protection, and Land authorities for the purpose of preparing and/or evaluating scientific experimental projects.

Since 1992, ZEBET has been offering, in collaboration with the Freie Universität Berlin and the Humboldt University Berlin, seminars and internships for natural scientists, biologists, physicians and veterinarians who are responsible for supervising animal experiments or conduct such experiments themselves. The aim of this training is to impart general knowledge about the regulations governing animal protection as well as special knowledge about refining existing experimental methods using animals, which leads to the replacement and reduction of animal experiments. The training offered is in line with the international recommendations which apply in this area.

This was only a brief outline of the wide range of activities carried out by ZEBET. It manages, however, to convey to you some impression of how successfully this institution has been able to develop since its inception. I would like to take for myself this opportunity to thank all of those responsible for this great achievement and to express the hope that this successful work for the benefit of animal welfare will continue in the future.

Animal protection policy

Although the Federal Republic of Germany is among those countries of the European Union with the most stringent legal provisions on animal protection, I do believe that there is still room and need for improvement. The new Federal Government acknowledges what has already been achieved but would like to award an even higher status to animal protection than has hitherto been the case. It is our declared objective to pursue the aims of animal protection both nationally and at European level in a decisive manner by means of appropriate legislative measures, combined with European initiatives.

One very significant national project in this area is the initiative, enshrined in the coalition agreement of the new government, to include animal protection in the Basic Law. We believe that it is necessary to establish animal protection in the Constitution so as to make it possible to balance animal protection with the other

rights protected under it, in particular with the basic rights.

Corresponding bills of law dating from the preceding legislative period have been re-introduced and are currently being discussed in the competent Committees of the German Bundestag. Under discussion is not only the bill tabled by the parliamentary groups composing the coalition, but also bills submitted by the Liberal (FDP) parliamentary group, the PDS and the Bundesrat.

I am well aware of the fact that massive reservations against establishing animal protection in the Basic Law are harboured by scientists and their organisations. Their concerns range from the limitation of the freedom of research all the way to an almost total standstill in research and the jeopardisation of Germany's competitiveness as a scientific and economic location. Similar arguments were also put forward at the founding of ZEBET. Today, on the 10th anniversary of its inception, the opposition has long subsided.

I hope that the successful and trustful process of enforced collaboration of all different groups concerned will be helpful in the debate about animal rights.

I would like to make it quite clear that it is not a question of invalidating the possibility of conducting animal experiments by introducing animal protection as a fundamental aim of state policy. Instead, our intention is to award animal protection a higher status than it now enjoys. A series of recent court decisions evidence the fact that animal protection always takes second place when it comes into conflict with basic rights which enjoy unrestricted protection. We would like to secure for animal protection equality of opportunity in principle. In this endeavour we can also count on the backing of the people we represent since corresponding survey results show that the great majority of the population is in favour of establishing animal protection in the Basic Law.

Against this background, and in the light of the broad support which this matter has received in Parliament, the majority required for a constitutional amendment to include animal protection in the Basic law is now within reach. The task now before us is to arrive at a comprehensive consensus which takes account of all the interests involved. On the one hand, the concerns of animal protection should be taken into account to the greatest possible extent and, on the other, it should be made clear that there is a need for a balance between that and other rights protected by the Constitution.

Animal experiments

We always find ourselves drawn in a difficult process of weighing the protection of human health against animal protection. I am aware that, according to current scientific knowledge, it is not possible to forgo animal experiments on a general basis. However, this circumstance also obliges us to undertake all that is in our power to continue to reduce the number of laboratory animals necessary and, at the same time, the suffering they are made to undergo. We must foster the development of alternative and supplementary methods and push forward their acceptance and implementation. Since the great majority of the animal experiments necessary for the marketing of products is based on international regulatory provisions or agreements, it is especially at international level that the convincing has to be done.

The past few years have shown that this is indeed a long and difficult process. Nevertheless, on the basis of the figures at our disposal, we can assert that we are on the right track. Germany has witnessed a constant decline in the use of laboratory animals since 1989, when official data compilation was introduced. Between 1991 and 1997, the number of laboratory animals used dropped from 2.4 million to just under ~.5 million. It is especially in the area of the development and testing of pharmaceutical products that it has been possible to achieve a steady reduction in the number of laboratory animals required every year. This is most likely due, to a major extent, to the increasing use of *in vitro* methods for the development of new active ingredients.

Closely connected with the European efforts to improve the protection of animals used for experimental purposes, is also the draft of an amended ordinance on the compilation of the necessary data bearing on the use of the vertebrates currently needed for scientific purposes. This draft, which has been transmitted by the Federal Government to the Bundesrat, is intended to increase transparency in this field and thereby address one of the urgent concerns of those members of the public who are interested in animal welfare. Furthermore, the new concept also takes account of the agreement adopted at EU level which places its main emphasis on the compilation of detailed information on those animal experiments which are prescribed by law. This more or less brings us back full circle to alternative and supplementary methods.

In order to bring about advances and improvements in the housing and keeping of animals for experimental purposes, the Federal Government plans to undertake intensive efforts at the level of the Council of Europe. In fact, the up-dating of the corresponding recommendation is currently under discussion there.

Animal experiments and cosmetics

The public takes an especially critical view of the use of animal experiments for the development of cosmetics. Although the use of animal experiments for the purpose of developing cosmetics is prohibited in Germany and in the Netherlands, only a regulation at Community level will be able to address the need for effective animal protection.

At least, in 1993 the EU succumbed to German insistence and incorporated a ban on the placing on the market of cosmetic containing ingredients or combinations of ingredients which have been tested on animals. The marketing ban also covers third countries and is to enter into force on 30th June 2000.

The basic problem in this area was and continues to be the fact that the fulfilment of health safety requirements in the manufacture and marketing of cosmetics must be ensured even when taking animal protection concerns into consideration.

There is a great deal of work still to be done here and what lies ahead can even be depicted in figures. The EU inventory for cosmetics contains over 6,500 substances and approximately 2,500 fragrances and aromatic substances. These substances need to be tested time after time to determine whether they are safe according to the most up-to-date scientific findings.

As a result of the afore-mentioned problems of a fundamental nature, the EU's marketing ban has been issued with the proviso that altematives to animal experiments based on OECD guidelines must be available.

The development by ECVAM and ZEBET of alternative test procedures to replace animal experiments therefore acquires special significance. However, the three methods validated thus far are still awaiting world-wide recognition by the OECD. Additional alternative methods for the testing of substances, for example with regard to toxic and teratogenic effects, remain elusive.

We would also be making a step in the right direction by stipulating that alternative methods for the testing of ingredients used in cosmetics have to be applied immediately in the Member States as soon as they are developed and validated by ECVAM. It would be indefensible for us to continue to wait until the lengthy OECD validation procedures are finally completed.

At any rate, despite enormous efforts on the part of all of those concerned, it has been possible to develop and validate the necessary alternative methods which can be utilised in the health-related evaluation of cosmetics only in some areas. In addition, the EU Commission fears that the entry into effect of the product-related ban could be in conflict with international trade legislation since products from countries such as the USA and Japan, which are not likely to dispense with animal testing in the foreseeable future, would not be marketable within the Community from 30th June 2000 onwards.

As a result, the EU Commission now intends to abandon the ban contained in the Cosmetics Directive on the marketing of the affected cosmetics and to insert a clause in the directive banning the testing of finished products on animals in its place. Animal experiments for the testing of cosmetic ingredients are to be prohibited gradually as soon as corresponding alternative methods have been validated by ECVAM.

This is why I sincerely hope that the new EU Commission will soon resume work on the Directive to Amend the Cosmetics Directive and that, as far as possible, the envisaged regulations will also take account of animal welfare concerns. The right kind of signal would also be given if the ban on animal experiments in the development of cosmetics which is currently in force in Germany were to be introduced for the entire European Union.

Research for the benefit of animal protection

Research is undertaking enormous efforts to find alternative methods to animal experiments. In addition to the contribution which ZEBET is making in this field, there is a series of additional activities which deserve to be mentioned here.

For example, as early as 1980, a priority area for funding - "Alternative Methods to Animal Experiments" - was established at the Federal Ministry for Research. This measure is the only one of its kind and the most expensive state-sponsored measure with this objective in the world. Between 1980 and the close of 1997, some 127 million Deutsche Marks in funding was awarded by the Federal Ministry for Research and Technology. For 1999 and the year 2000, approximately 9.5 million Deutsche Marks per annum will be available. This funding has already made it possible to lay the foundations for achieving considerable reductions in the number of laboratory animals necessary in many areas.

As early as 1986, the Federal Government supplemented this process by setting up the Foundation to Promote the Research into Alternative and Supplementary Methods and to Reduce Animal Experiments (set) together with associations from the industrial sector and animal protection organisations. The Foundation funds research projects on the reduction of animal experiments and provides an excellent example of successful collaboration between industry and animal protection organisations. In the past 8 years, it spent approximately 3.7 million Deutsche Marks funding the various projects.

Federal Ministry for Health Research Prize

Another contribution to animal protection is a research prize which the Federal Ministry for Health has been offering on an annual basis since 1991. The prize to "Promote Methodological Work Aimed at Reducing and Replacing Animal Experiments" is worth 30.000 Deutsche Mark. It is awarded for scientific works which make a contribution particularly to the further development of pharmacotoxicological test methods. The prize is awarded on the recommendation of an independent committee comprised of representatives from the fields of science, business and animal protection.

A range of scientists have once again submitted their work for the 1998 Research Prize. The 1998 research prize of the Federal Ministry for Health is to be awarded today to a young scientist, Dr. Siegel-Axel. I hope that the day will come when the achievements and successes of women in science do not require special mention.

Dr. Siegel-Axel developed a standardised model for routine pharmacological pre-screening procedures in arteriosclerosis research, thus opening up the possibility of reducing the number of animal experiments in research in this field. I prefer to leave all further explanation of her work to the expert herself - today's award-winner.

To you Dr. Siegel-Axel, I would like to extend my warmest congratulations and at the same time take this opportunity to wish all of the participants in the Symposium two very successful, interesting days here in Berlin.

National Co-operation

Brigitte Rusche
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ZEBET: Institutionalised co-operation between animal welfare, industry and the government

Summary
In Germany, more than 10 years ago, there was agreement between the industry and the animal welfare movement that a focal point is needed, in particular on national, governmental level, to promote the use and the application of alternative methods. Together with remarkable support from the German government this agreement has been transformed into an institution that since its foundation in 1989 has received a world-wide reputation and acknowledgement from all interest groups involved: ZEBET.
Of course, ZEBET is not an animal welfare organisation. However, at ZEBET the opinions of animal welfare representatives are respected and taken into account via a formal route which is essential for this institution. From the beginning, the animal welfare community was involved in the organisation and operation of ZEBET, and it still is: Animal welfare representatives participate in ZEBET's Scientific Advisory Committee - just as representatives from industry and the government.
This open communication platform is one of the main reasons why there is constructive co-operation between animal welfare, industry, and the government at ZEBET in spite of sometimes conflicting positions of these interest groups. This collaboration has proven to be fruitful in many ways. What is still needed is another type of co-operation: ZEBET's obligatory involvement in any decisions relevant to the field of animal experiments and alternatives. More than in the past ZEBET should be given the role of a co-ordinating agency e.g. for other governmental institutions concerned with problems of consumer or environmental protection.

Zusammenfassung: Institutionalisierte Zusammenarbeit zwischen Tierschutz, Industrie und Regierung
Vor mehr als 10 Jahren gab es in Deutschland zwischen der Industrie und der Tierschutzbewegung Übereinstimmung darin, daß insbesondere auf nationaler Regierungsebene eine zentrale Anlaufstelle zur Förderung der Entwicklung und Anwendung von Alternativmethoden benötigt wird. Auf Grund der bemerkenswerten Unterstützung der deutschen Regierung konnte eine Einrichtung geschaffen werden, die seit ihrer Gründung im Jahre 1989 weltweiten Ruf und die Anerkennung aller involvierten Interessensgruppen erhalten hat: ZEBET.
Natürlich ist ZEBET keine Tierschutzorganisation. Dennoch werden bei den Aktivitäten von ZEBET die Ansichten von Tierschutzvertretern berücksichtigt und ernst genommen - und zwar auf eine für diese Einrichtung essentielle Art und Weise. Von Anfang an war die Tierschutzbewegung bei der Einrichtung und Funktion von ZEBET involviert, und sie ist es immer noch: Tierschutzvertreter arbeiten in der ZEBET-Kommission ebenso mit wie Repräsentanten von Industrie und Regierung. Diese offene Kommunikationsplattform ist einer der Hauptgründe dafür, weshalb es trotz oft grundlegend unterschiedlicher Positionen eine konstruktive Zusammenarbeit zwischen Tierschutz, Industrie und der Regierung bei ZEBET gibt. Diese Zusammenarbeit hat sich in vieler Hinsicht als fruchtbar erwiesen. Was immer noch verbesserungsfähig ist, ist eine andere Form der Zusammenarbeit: ZEBET's obligatorische Einbeziehung in jegliche Beratungen und Entscheidungen, die für das Gebiet der Tierversuche und Alternativmethoden von Relevanz sind. Mehr noch als in der Vergangenheit muß hier ZEBET die Rolle einer zentralen koordinierenden Stelle zugesprochen werden, zum Beispiel für andere behördliche Einrichtungen, die sich mit Problemen des Verbraucher- oder Umweltschutzes befassen.

Keywords: 3R, animal welfare, national centre, communication, co-ordination

ZEBET's history

In Germany, in the 80s, there was agreement between the pharmaceutical industry and the animal welfare movement that some kind of a focal point was needed to promote the development and application of alternative methods.

Despite some critical voices the decision was made to set up a national alternatives centre. The former president of the Bundesgesundheitsamt (German Federal Health Agency), professor Großklaus, who personally was committed to this issue, communicated intensively with the various interest groups and finally set up an appeal tribunal with representatives from the government, industry, academia and animal welfare.

The appeal tribunal's joint recommendation to appoint Professor Horst Spielmann director of ZEBET was then accepted by the responsible authorities.

That was a good basis for a trusting co-operation between ZEBET and the interest groups involved and this basis was further strengthened by the installation of the ZEBET Committee where these interest groups were given a forum for information, discussions and recommendations.

One of the main interests of animal welfare organisations in Germany is the abolition of animal experiments.

The interests of animal welfare and the relevant industry meet when scientific methods that are not based on animal experiments lead to the goal faster, cheaper, and safer than *in vivo* methods or when animal experiments can be deleted from official requirements because it is evident that they do not provide a reasonable or significant contribution - this is for instance the case for abnormal toxicity testing.

Regarding the different states of new methods in different areas of science either the development of a new method, or its standardisation, validation, or acceptance are the necessary steps to be taken. In all of these tasks ZEBET as a governmental institution plays an essential role.

Co-operation with SET

Therefore it is very much welcomed that ZEBET is actively contributing to the work of SET - the Foundation for the Promotion of Research on Replacement and Complementary Methods to Reduce Animal Testing (Stiftung zur Förderung der Erforschung von Ersatz- und Ergänzungsmethoden zur Einschränkung von Tierversuchen) - in which industry and animal welfare work together with representatives from the Federal Government, churches and labour unions.

Co-operation with animal welfare organisations

People from animal welfare are highly motivated to look for new possibilities to save animals, to take part in intensifying efforts to replace animal experiments or to promote the political framework. Looking back ten years the co-operation between ZEBET and the animal welfare movement has proven to be very fruitful which can be demonstrated by the following examples.

At an early stage of the functioning of ZEBET the need for a far-reaching involvement of ZEBET into any decisions relevant to the field of animal experiments and alternatives became evident. There were plans within the German Institute for Standardisation (Deutsches Institut für Normung, DIN) to introduce primate experiments for the testing of dental materials even though sound alternative methods for that purpose existed. There were strong protests against these plans from the animal welfare movement. As a consequence thereof, ZEBET as well as representatives from animal welfare were given the opportunity to participate in the respective DIN working group. Since then, substantial contributions could be made with regard to the application of alternative methods. The importance of taking into account animal welfare considerations at this level has been stated again in the last meeting of the ZEBET commission and it becomes evident from the fact that norms often become a part of legal documents.

There were several national and international symposia organised by animal welfare that gained importance not at least thanks to the fact that ZEBET was involved. For example as a result of a symposium in Poland, financially supported by the foundation SET, the Polish government is now on the way to establish its own national alternative centre.

There was a positive co-operation between animal welfare and ZEBET in establishing databases, and ZEBET's database has become a helpful instrument not only to scientists or authorities but also to members of ethical committees to decide on the ethical justification of research proposals involving the use of living animals.

Due to the fact that the Deutsche Tierschutzbund has a cell culture laboratory located in its Akademie für Tierschutz there was also successful co-operation with ZEBET in the field of validation of cell culture tests.

Further improvements are essential

However, there are some points in which further improvements are essential from the point of view of animal welfare. In the past years we have made the experience that timely intensive communication and support cannot be taken for granted between the different responsible governmental institutions and even sometimes within the bgvv, Federal Institute for Health Protection of Consumers and Veterinary Medicine, which, as we know, is affiliated to ZEBET.

An example of lacking communication and co-ordination is the issue of endocrine disrupters: Even though according to the director of ZEBET and a number of experts from industry and academia essential preconditions are lacking for the introduction of animal tests for the routine testing on endocrine disrupting activity of substances (such as scientific evidence for the assumed risk as well as for the underlying mechanisms) we were told by the German Minister of the Environment that his experts hold a different view and that he would rely on their expertise.

These experts even supported the introduction of an OECD Guideline for an uterotrophic screening assay, the „Immature Rat Utereotrophic Bioassay Screening Test", even though this assay is based on an obsolete animal test that has been invented some 60 or 70 years ago. Similar problems exist with regard to ECVAM at the EU-level.

ZEBET needs more staff

There should be agreement between all of us that whenever new testing methods, guidelines etc. in the name of consumer, animal or environmental protection are discussed it is necessary from the very beginning to reflect the present knowledge on advanced methods, and, which is mostly neglected, to start looking for new methods. And, if existing methods are reviewed, this special knowledge on alternatives must be given full consideration.

That means that more personnel has to be conceded to ZEBET. In fact the unsatisfactory situation at ZEBET with regard to staffing has been on the agenda of animal protectionists and others for a long time. Even the Petitionsausschuß des Bundestages (petition committee of the German Parliament) emphasised the fact that for a proper functioning of ZEBET its staff would have to be expanded at least to the number of posts that had been planned from the beginning and which was regarded modest anyway.

The petition committee also stated that any reduction in the previously planned posts would undermine the political will of all parties of the German Parliament. That was in 1990. Nothing positive has happened since then in this respect - the opposite is the case: ZEBET's staff has been further reduced whereas its area of responsibility has grown.

The animal welfare community would like to have ZEBET as a powerful institution that is equipped accordingly and as a communication centre in order to save as many animals as possible from the fate of becoming an experimental testing system.

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ZEBET and the cosmetic industry: a decade of national and international co-operations

Summary

An overview is given on the co-operation between ZEBET and the cosmetic industry both in Germany (IKW) and Europe (COLIPA). The co-operation began in 1988 in the German „BGA-Studie" on alternatives to the Draize rabbit eye irritation test and was continued in the European validation study on alternatives to the Draize eye test, the EC/HO-Study. Other joint validation activities are briefly reviewed. Finally, results of the successful EU/COLIPA validation study on in vitro *photoirritation test are presented, which lead to the validated 3T3 Neutral Red Uptake Phototoxicity Test (3T3 NRU PT), which was accepted for regulatory purposes in 1997/1998 both by EU experts (ESAC) and regulatory authorities (DG III, IX, and XXIV).*

Zusammenfassung: ZEBET und die Kosmetikindustrie: Eine Dekade mit nationalen und internationalen Kooperationen zur Validierung von Ersatzmethoden zum Tierversuch
Während der vergangenen Dekade haben sich die deutsche und die europäische Kosmetikindustrie in verschiedenen, teils sehr erfolgreichen, Projekten zusammen mit ZEBET um die Validierung von Alternativmethoden zum Tierversuch bemüht. Die wichtigsten Fragestellungen betrafen die Methoden zur lokalen Verträglicheit, wie z.B. der Augenschleimhautreizung und der Phototoxizität topisch applizierter kosmetischer Zubereitungen, bzw. deren Inhaltsstoffe und die daraus resultierenden Projekte.
Das erste gemeinsame Projekt fiel in die Gründungszeit von ZEBET und ist unter dem Titel "bga–Studie" bekannt. In diesem vom BMFT unterstützten Projekt wurden der HET-CAM und der NRU-Test als Alternativen zum Augenschleimhautreiztest am Kaninchenauge entwickelt und geprüft. In einem biometrischen Folgeprojekt ergab sich aus dem umfangreichen Datenmaterial ein erfolgversprechender Ansatz zur Identifizierung stark reizender Stoffe, die gemäß der EU-Regulierung mit R41 zu klassifizieren sind.
Die Erfahrungen aus diesem Projekt flossen unmittelbar über ZEBET in die Planung einer ersten internationalen Studie ein, die unter dem Titel "EC/Home Office Study" bekannt und publiziert wurde.
Parallel zu diesem Projekt startete die europäische Kosmetikindustrie – vertreten durch ihren Verband COLIPA – ein analoges Projekt, das auf die speziellen Bedürfnisse und Anwendungen maßgeschneidert war.
*Zur selben Zeit startete COLIPA zusammen mit der Europäischen Kommission und ZEBET als Subkontraktor ein Validierungsprojekt mit dem Titel „*Phototoxicity in vitro*". In einer Prävalidierungsphase wurden relevante Methoden untersucht, die zur Validierung geeignet und „reif" erschienen. Unter diesen Methoden fiel der von der Kosmetikindustrie entwickelte „Neutral Red Uptake Phototoxicity Test" durch seine hohe Prädiktivität auf. Die Validierungsstudie wurde ebenso wie die Prävalidierung erfolgreich vom Management Team unter Federführung von ZEBET koordiniert. Die Ergebnisse dieser Studien sind dann in erfolgreicher Kooperation von COLIPA und ZEBET dem SCCNFP nahegebracht worden, so daß die Methodik nach ihrer wissenschaftlichen Akzeptanz durch ECVAM und sein wissenschaftliches Beratungsgremium zu einer der ersten akzeptierten Ersatzmethoden wurde, für die nunmehr ein Entwurf bei der OECD vorliegt.*
Zusammenfassend kann die konstruktive Zusammenarbeit zwischen ZEBET und der europäischen Kosmetikindustrie an dieser Stelle nur nochmals lobend hervorgehoben werden.

Keywords: 3R, cosmetic industry, validation process, co-operation with ZEBET

Introduction

About more then ten years ago, when co-operation between industry, in particular cosmetic industry, and German authorities started, the use of animal testing for consumer safety reasons was often under discussion in the public domain. On the one side official guidance documents existed and on the other side few years later animals tests were *de facto* legally banned. At the same time the German initiative started as a large evaluation programme using the Hen's Egg Test on the Chorio-Allantoic Membrane (HET-CAM) and the Neutral Red Uptake Test (NRU-Test) established and performed in 14 laboratories from academia and industry. The project was financially supported by the Federal Ministry of Research and Technology and was followed by a post-hoc biometrical project to analyse the huge amount of data.

At the European level this project was followed by the UK Home Office initiative named EC/HO-Study conducted by ECVAM and its management team with ZEBET as a member from the authorities' side. This study was closely linked to the COLIPA project on alternatives to animal testing dealing with methodologies developed to replace the Draize eye irritation test an initiative of the steering committee on alternatives to animal testing (SCAAT).

In those days SCAAT has initiated a second project dealing with phototoxicity testing *in vitro* and as an answer to an OECD proposal on dermal photoirritation testing, in order to avoid the international establishment of a new guideline using animals in this area of chemical hazard identification and risk assessment.

The second project on *in vitro* phototoxicity testing led us to the successful validation and implementation of the Neutral Red Uptake Phototoxicity Test on fibroblasts in the EU and its proposal as the first OECD Guideline using *in vitro*

methodology once it will be internationally accepted.

Beside of other activities ZEBET was engaged and successfully co-operating in all these projects on alternatives to animal testing during the ten years of existence.

German research projects on alternatives to eye irritation *in vitro*

A precursory project delt with the optimisation and development of the two selected test methods: the HET-CAM and the NRU Test in two laboratories. The promising outcome of this first project, a sort of test development project, led to the planning of a large experimental validation and evaluation programme (bga-Studie) with 14 laboratories from industry and academia performing one or both tests after their implementation in the laboratories and the training of the technicians. This experimental phase itself was divided in three parts. The first organised as a training and implementation phase testing some few chemicals and a second phase testing about 50 chemicals to prove reliability aspects and to correlate *in vitro* data to *in vivo* assessments. During the third phase the number of laboratories was reduced to seven and the quality of *in vivo* data was increased. Only chemicals with corresponding actual EU risk phrase classifications were accepted for this experimental validation process.

Finally, the programme was followed by a project sponsored by the Federal Ministry of Research and Technology as well, to perform a post-hoc biometrical analysis to find appropriate ways to interpret the data and to optimise the outcome of this outstanding programme.

During the initial phase of the programme ZEBET was born and Horst Spielmann, who became head of ZEBET, and his colleagues co-ordinated the whole project. A number of lessons have been learned during the work. Criticism came in particular from outside, although at the end a combination of data from both methods could discriminate chemicals classified as R41 from less irritant materials.

The EC/HO-study – an international validation project

In order to overcome deficiencies of the German project and to organise an international formal validation study ECVAM started the EC/HO-project with nine selected *in vitro* methods representing different types of experimental approaches, like biophysical measurements, cellular tests and organotypic models, each performed in four laboratories including one as lead laboratory. The major goal of this study testing 60 well selected chemicals with high quality *in vivo* data was to identify those test methods able to discriminate either only the very strong irritants or even to replace the whole eye irritation test on rabbits.

The outcome showed that none of the methods were able to meet the goal, but some methods seem to be appropriate to predict irritancy of certain classes of chemicals, for instance, like surfactants which behave in a similar physico-chemical type of interaction with tissue and cellular components like bio-membranes and proteins. Both types of reactions are known to play an important role primary eye tissue damage.

Other activities in this context

A major problem of this project was the appropriate evaluation of the large amount of experimental data, in particular since the methods involved were not able to predict *in vivo* irritancy, expressed as classifications of irritancy or modified mean average scores of ocular tissues. This drawback should be overcome in a COLIPA initiative which was closely linked to the EC/HO-Study, not only with an overlap in test protocols, but also with chemicals and participating laboratories. The essential difference was the implementation of prediction models in order to make the prove of relevance more easy. Some tests were able to predict acute eye irritancy of finished formulas better than for chemicals as used as ingredients in cosmetics.

One test which was developed on the base of common molecular mechanisms and interactions between surfactants and cellular structures looks among others quite promising. This test, the Red Blood Cell Test (RBC test), was in a German ring trial with several surfactants producing companies supported by ZEBET.

Phototoxicity testing *in vitro* – a success story

Parallel to the various activities in the area of alternatives to eye irritation testing in the rabbit eye, COLIPA and the European Commission has started a project on phototoxicity testing *in vitro* in 1991.

This initiative started with collecting information from in house practice of phototoxicity test of some few companies and ends up with the planning of an experimental programme, in order to evaluate the different methodologies, of which some had already a long history in testing at cellular level, such as the Photo RBC Test for testing photodynamic effects as already published in 1904. Prior to the beginning of the experimental work it was decided by the COLIPA Task Force on Photoirritation *in vitro*, which was the expert panel, to use one common test protocol in each participating laboratory as a sort of internal standard or benchmark, beside various different in house protocols as practised in the laboratories in daily routines.

This common protocol was easy to implement in the other laboratories because it was based on the Neutral Red Uptake test in 3T3 mouse fibroblasts a widely used cytotoxicity method proposed by E. Borenfreund some years ago. The photocytotoxicity protocol was designed to cover most sensitive all relevant phototxic mechanisms at cellular levels, such as membrane damage by photodynamic reactions, oxidative damage of cellular proteins and DNA should be included as well as other light induced radical reactions and covalent binding processes to important cellular structures by direct action of mainly UVA and visible light on phototoxins incubated with the cells. The protocol also implies an agreed common sun simulator as light source, which had to be used in each laboratory to avoid the application of different lamps reflecting different philosophies in photobiology.

The experimental approach in Phase I – organised as pre-validation step – was a success. ZEBET was asked to co-ordinate all activities during this phase including the first ECVAM workshop on Phototoxicity Testing *in vitro*.

Therefore this project could and can be considered as the first successful co-operation between in particular the cosmetic industry and ZEBET, which was under the contract of ECVAM. The second phase – designed as a formal validations study under blind conditions with independent

sample supply and biostatistican confirmed the good outcome of phase I and the prediction model proposed. This international validation study was of particular importance, because the successful method was considered to be the first relevant proposal of an *in vitro* method submitted for the OECD Guidelines for testing chemicals.

Finally, a study requested by the SCCNFP (Scientific Committee of Cosmetology and Non-Food Products) on UV-Filter substances as used in sunscreens was performed in a few experienced industrial laboratories again with good success and under the hospice of ZEBET and ECVAM.

Acknowledgement
Facing the constructive and engaged work done by ZEBET as co-ordinator of several validations studies mainly in the area of the search of alternatives for eye irritation and photoirritation, which means explicitly done by Horst Spielmann and his colleagues, and on the occasion of the 10th anniversary of ZEBET the European and German cosmetic industry represented by COLIPA and the IKW thanks for the excellent co-operation during these ten years.

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International Co-operation

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Symposium „Implementation of the 3R targets in the EU, in science and industry"

Abstract
During the Austrian Council Presidency in November 1998 the Federal Ministry for Science and Transport (BMWV) organised a symposium „Implementation of the 3R targets in the EU, in science and industry". The aim of this symposium was to establish for the first time a discussion forum for the EU and national funding agencies, universities, industrial partners and scientists. The programme reflected the diversity in the field, namely the European Commission and the OECD itself in its diversity, 3R priorities in the industry and information systems, views and trends from national authorities as well as scientific perspectives and future trends. More than 230 participants from 19 countries contributed to the success of the symposium.
A one page résumé and conclusion was finally accepted and forwarded to the Environmental Council:
„The European Commission should encourage the international acceptance of alternative methods validated and used within the EU, and should catalyse European industrial and academic activity by developing collaborative programmes on alternatives research. The European Commission (DG XI and the JRC) should play a pivotal role in co-ordinating these 3R-related activities in the Member States, and in particular through ECVAM, which was established for this purpose.
The European Commission should implement a pan-European, multinational discussion forum to foster debate on real and potential opportunities for the 3Rs, and should strive to exert greater impact in global debates, especially involving the OECD, the USA and Japan.
The role of ECVAM in developing and validating replacement alternatives should be improved and encouraged. In particular, ECVAM should be given sufficient resources and a widened responsibility for co-ordinating and financing research and the development and validation of scientifically advanced methods in the field of alternatives in the EU.
The present information services from ECVAM should be further developed and improved, to become a europe-wide database for scientific information concerning alternatives to laboratory animal procedures. Making the information available would be best achieved via Internet. The databases of different countries should be better linked to each other via ECVAM. A world-wide information network should be established.
All governmental funding agencies in the area of Life Sciences, both of the European Commission and of the Member States, should apply the 3R principles as an essential criterion in evaluating grant applications.
Academic researchers should be made more aware of the 3R principles and should be encouraged to focus more on replacement alternative methods and their validation, as well as on reduction and refinement. They also should be encouraged to access and to use the established databases."
These conclusions were then forwarded to the commission. The German presidency as well as the following presidencies were kindly asked to follow up the important issue of animal testing and its alternatives.

Keywords: 3R, EU, European Commission, ECVAM, OECD

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ECVAM and the promotion of international co-operation in the development, validation and acceptance of replacement alternative test methods

Abstract
The European Centre for the Validation of Alternative Methods (ECVAM) was established by the European Commission in 1991, to co-ordinate the validation of alternative methods at the European Union level, to establish a database on alternative methods, and to promote dialogue among all the interested parties, in order to secure the international recognition and acceptance of validated alternative test methods.
All ECVAM's activities, including workshops, task forces, in-house laboratory studies, and contracted external pre-validation and validation studies, involve international co-operation.
Of particular importance has been the role played by ECVAM, notably in partnership with ZEBET in establishing the principles of test development, validation and acceptance, defining the practical procedures necessary to optimise the progress of new tests toward acceptance, and in successfully validating the 3T3 NRU PT test for phototoxic potential.
It will be concluded that it is vital that international co-operation goes beyond mere discussion and leads to definite collaborative projects, which lead, in turn, to genuine achievements.

Keywords: 3R, ECVAM, ZEBET, validation studies, co-operation

Kurzfassung: ECVAM und die Förderung internationaler Zusammenarbeit bei der Entwicklung, Validierung und Akzeptanz alternativer Testmethoden
Das Europäische Zentrum für die Validierung von Alternativmethoden (ECVAM) wurde von der Europäischen Kommission 1991 gegründet, um auf EU-Ebene die Validierung von Alternativmethoden zu koordinieren, eine Datenbank für Alternativmethoden einzurichten sowie den Dialog aller interessierten Organe zu fördern und um die internationale Anerkennung und Akzeptanz alternativer Testmethoden zu sichern.
Alle Aktivitäten von ECVAM, d.h. Workshops, spezielle Arbeitsgruppen, eigene Laborstudien und vertragliche, externe Prävalidierungs- und Validierungsstudien beruhen auf internationaler Zusammenarbeit.
Von hervorragender Bedeutung war ECVAM's Rolle - besonders auch in der Partnerschaft mit ZEBET - bei der Erarbeitung von Prinzipien für die Testentwicklung, Validierung und Akzeptanz und bei der Definition der notwendigen Schritte zu einer Optimierung der neuen Tests im Hinblick auf deren Akzeptanz. Dank dieser Vorarbeiten verlief die Validierung des 3T3 NRU PT Tests zur Prüfung des phototoxischen Potentials erfolgreich.
Es ist sehr wichtig, dass die internationale Zusammenarbeit über blosses Diskutieren hinauskommt und stattdessen konkrete, gemeinsam durchzuführende Projekte definiert werden, die dann ihrerseits echte Erfolge erzielen.

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Validation and regulatory acceptance of alternative test methods and international co-operation

Summary
The U.S. Food and Drug Administration (FDA) is committed to facilitating the development, validation, and regulatory acceptance of new toxicological testing methods. FDAs mission emphasizes protecting public health by using the best science through the most efficient means available. New technologies currently under development have the potential to better predict human and animal endpoints in safety assessment, and to reduce, refine, and replace animal use in product testing. Tremendous progress in the alternatives arena has been made and includes: (1) international harmonization on validation criteria for new test methods, (2) development of a process for the review and validation of new test methods by the European Centre for the Validation of Alternative Methods (ECVAM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), and (3) successful regulatory acceptance and implementation of new validated methods in toxicity testing. There are many participants to be commended for their accomplishments on these complex issues. However, there is much to be done, and continued cooperation between stakeholders internationally is essential. Moreover, mutual agreement must be reached regarding the validation status of those methods subjected to rigorous, expert peer review by organizations such as ECVAM and ICCVAM. International harmonization, based upon sound scientific principles, will prevent duplication of scarce resources, minimize unnecessary use of animals, facilitate safety assessment and regulatory acceptance, and ultimately reduce research-to-market time for regulated products.

Keywords: 3R, international harmonization, validation, regulatory acceptance

Zusammenfassung: Validierung und behördliche Anerkennung von Alternativmethoden in den U.S.A. - internationale Zusammenarbeit

Die U.S. amerikanische Food and Drug Administration (FDA) ist verpflichtet, die Entwicklung, Validierung und behördliche Anerkennung neuer toxikologischer Prüfmethoden zu fördern. Die vordringlichste Aufgabe der FDA, die Öffentlichkeit vor gesundheitlichen Risiken zu schützen, lässt sich am besten bewerkstelligen, indem die beste Wissenschaft mit den effektivsten zur Verfügung stehenden Methoden angewendet wird. Neue Technologien, die gerade entwickelt werden, haben das Potential, Endpunkte in der Sicherheitsprüfung bei Mensch und Tier besser vorherzusagen und so die Verminderung von Belastungen nach dem 3R Prinzip bei der Produktkontrolle zu gewährleisten. Es wurden enorme Fortschritte auf dem Gebiet der Alternativmethoden gemacht; sie umfassen (1) die internationale Harmonisierung von Validierungskriterien für neue Testmethoden, (2) die Entwicklung eines Review-Verfahrens für neue Testmethoden bei ECVAM und ICCVAM und (3) die erfolgreiche behördliche Anerkennung und Einführung von neu validierten Methoden bei der Toxizitätsprüfung. Vielen Teilnehmern an diesen komplexen Verfahren muss für ihre Mithilfe gedankt werden. Es gibt jedoch noch viel zu tun, und die internationale Kooperation zwischen den wichtigsten Zentren ist wichtiger denn je. Darüber hinaus muss die gegenseitige Anerkennung der Validierung von Methoden erreicht werden, die bei Organisationen wie ECVAM und ICCVAM einer strengen Expertenprüfung unterworfen wurden. Eine internationale Harmonisierung, basierend auf vernünftigen wissenschaftlichen Prinzipien, verhindert den unnötigen doppelten Einsatz der ohnehin knappen Mittel, minimiert die unnötige Verwendung von Tieren, erleichtert die Sicherheitsprüfungen und die behördliche Zulassung und reduziert so letztlich die Zeit, die von der Entwicklung bis zur Zulassung genehmigungspflichtiger Produkte vergeht.

Introduction

The title of this presentation is intended to convey three concepts that illustrate significant progress that has been made in the past decade in the alternatives arena. It is safe to say that significant advances have been made in understanding the steps necessary to successfully move a new method from research and development to practical use in the regulatory community. Achieving validation, regulatory acceptance, and finally, international cooperation on the intended use of new testing methods constitute three exceedingly difficult and complex challenges. It is important to note that ZEBET has been extremely active and key to the tremendous progress that has been made not only in the European Union, but internationally, as well.

The Interagency Co-ordinating Committee on the Validation of Alternative Methods (ICCVAM)

In the U.S., we have been successful in developing a standardized approach for determining the validation status of a new method for the specific purpose of regulatory consideration. This model for validation and regulatory acceptance has been developed by ICCVAM, the Interagency Co-ordinating Committee on the Validation of Alternative Methods. The WEB site for learning all about ICCVAM may be accessed at http://iccvam.niehs.nih.gov

ICCVAM resides in the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH), National Toxicology Program (NTP), Research Triangle Park, North Carolina. ICCVAM was established in May 1997, and consists of over 40 people participating from 14 federal regulatory and research agencies. In addition, the NIEHS/NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) has been established to administrate ICCVAM initiatives. NICEATM provides support to ICCVAM to facilitate scientific peer review and interagency consideration of new test methods of multi-agency interests. ICCVAM provides recommendations to participating agencies and promotes regulatory acceptance of those methods that have been reviewed through the ICCVAM process and declared to be valid for a specific purpose. It is important to emphasize that each member agency has the prerogative to use the method according to their testing requirements and are under no obligation to use ICCVAM-recommended methods. Federal statutes and their regulations vary considerably between and within agencies, therefore, it was determined that the acceptance and implementation of new test methods must remain flexible at the agency level.

The original *ad hoc* ICCVAM was established as a result of a U.S. Congressional mandate, Public Law No. 103-43, Section 1301, the National Institutes of Health Revitalization Act of 1993. The publication of *Validation and Regulatory Acceptance of Toxicological Test Methods* * represented a most significant initiative that culminated nearly four years of work involving multiple stakeholders and transcended numerous boundaries including 15 federal agencies, industry, academia, public interest groups, and the international community. The final report, which may be located at (http://ntp-server.niehs.nih.gov/htdocs/ICCVAM/ICCVAM.html), was accepted by all participating agencies.

ICCVAM's mission is to foster and coordinate issues throughout the federal government that relate to the development, validation, acceptance, and national/international harmonization of toxicological methods. The ICCVAM review process has been developed and used, to date, on two test methods. This process has several distinct elements with separate yet dependent functions; effective communication between these elements is imperative. The test sponsor can be any person, group, or organization that desires to have a method evaluated for its validation status. The first step in the ICCVAM process is for the test methods sponsor to contact NICEATM.

NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

NICEATM is located at NIEHS and consists of 3-5 government professional and

administrative staff augmented with appropriate contract support. NICEATM provides support to the ICCVAM to carry out ICCVAM-directed activities and accomplish the following:

- Assess the completeness of submissions and determine if there are sufficient data for test methods to undergo independent public scientific peer review;
- Arrange for scientific peer reviews;
- Organize expert panels and/or workshops to assess the validation status of a method or group of methods;
- Provide recommendations and results to research and regulatory agencies;
- Communicate with interested stakeholders and facilitate communication during the development and validation process with appropriate agencies; and
- Prepare, publish, and distribute reports and information about new test methods.

Once NICEATM has determined that adequate data are available to go forward, an ICCVAM Interagency Working Group is formed. Member agencies are asked for volunteers to participate in the Working Group; the intent is for the agency participants to be well informed and where possible experts in the field of science represented by the new method. The Working Group is charged with interacting closely with the sponsor and NICEATM to assure the submission has met the validation criteria as outlined in the ICCVAM publication, *Validation and Regulatory Acceptance of Toxicological Test Methods*. An ICCVAM Interagency Working Group will be formed for all proposed test methods and plays a pivotal role especially within FDA.

When FDA is asked for volunteers for a Working Group, the request is distributed to each of its centers, which are organized along product lines. That is, each center is responsible for regulating different products such as biologics, human drugs, animal drugs, medical devices, food additives, etc. As a result, the ICCVAM Working Group may have several FDA representatives. From a FDA perspective, the direct involvement of these individuals is vital to the final stages of regulatory acceptance and implementation should the ICCVAM process result in a recommendation to the agencies. Because these participants are involved in making regulatory decisions based upon data generated through the proposed test methods, they are well positioned to convey the benefits and limitations directly to those within each FDA Center responsible for reviewing applications for regulated products. To date, this paradigm has worked well on the implementation of the Murine Local Lymph Node Assay (LLNA) ** within the FDA. The ICCVAM Working Group also provides valuable information to NICEATM in identifying experts external to the Federal Government to participate on the Peer Review Panel (PRP).

Peer Review Panel (PRP).

The PRP is a Federal Advisory Committee that must comply with the U.S. Federal Advisory Committee Act (FACA). The PRP consists of expert scientists from academia, industry, government, and international organizations. This committee must comply with FACA, which includes disclosure and open meeting requirements. Fundamentally, this assures a transparent process, which requires all PRP meetings to be announced through the *Federal Register*, meetings open to the public, and opportunity for public comment as part of each meetings agenda. In addition, the U.S. Freedom of Information Act (FOIA) requires public disclosure of documents relevant to the preparation and review process. Proprietary data and personal information on Federal employees are exempt from FOIA. The Working Group is responsible for focusing the PRP on specific questions to be answered depending upon the sponsors intended use of the proposed method. In other words, has the sponsor submitted cogent data, from well-controlled studies, that substantiate relevance and reliability for a specific stated purpose? Upon reviewing the data, the PRP will make a recommendation to ICCVAM. Once approved, the ICCVAM recommendation is sent to the 14 member agencies.

ICCVAM recommendations

The ICCVAM recommendation is conveyed to the agencies from the director, NIEHS/NTP, Dr. Kenneth Olden, who requests the agencies to respond. In their response, the agencies are expected to indicate the acceptability of the proposed method and the steps taken to implement use of the method within the agency.

Advisory Committee on Alternative Toxicological Methods

Another important component of the ICCVAM process is the Advisory Committee on Alternative Toxicological Methods, each is composed of scientific experts from academia, industry, federal and state government agencies, public interest organizations, and the international community. This committee meets semiannually to review ICCVAM programs and provide advice on the activities of NICEATM. It is important, however, to remember that the ICCVAM process exists within a much larger alternatives arena where new testing methods are subjected to many scientific and political forces.

International harmonization

The flow of new toxicological methods is a dynamic process commencing with the early stages of research, development, and prevalidation and ending with regulatory acceptance and implementation of the validated test method. This continuum is dynamic and contains multiple, complex, and difficult steps involving diverse players. Imperative to the success of this model is international harmonization and participation from all relevant stakeholders. As ZEBET, ECVAM, OECD, ICCVAM and other major players endeavor to develop, validate, and incorporate new testing methods into regulatory decision-making, it is incumbent upon the entire international community to understand the different models that have emerged to accomplish similar tasks. Once this understanding occurs, appreciation and respect

* Validation and Regulatory Acceptance of Toxicological Test Methods, A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods, National Institute of Environmental Health Sciences, National Institutes of Health, U.S. Public Health Service, Department of Health and Human Services, NIH Publication No. 97-3981, March 1997.

** The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals/Compounds, Results of an Independent Peer Review Evaluation Coordinated by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program Center for the Evaluation of Alternative Toxicological Methods (NICEATM), National Institute of Environmental Health Sciences, National Institutes of Health, U.S. Public Health Service, Department of Health and Human Services, National Toxicology Program, NIH Publication No. 99-4494, February 1999.

will result in mutual agreement and acceptance. Both ICCVAM and ECVAM have been successful in validating and achieving regulatory acceptance of proposed new test methods. ZEBET and FDA must share in the success of these organizations because they have demonstrated a strong commitment to promoting alternatives to traditional animal models.

Present work

The following test methods are currently under ICCVAM review:

- Murine Local Lymph Node Assay (LLNA) for detecting allergic contact dermatitis (hypersensitivity);
- Corrositex for dermal corrosivity; and
- Frog Embryo Teratogenesis Assay on *Xenopus* (FETAX), a developmental toxicity screening method.

The ICCVAM peer review of the LLNA and Corrositex methods has been completed and recommendations have gone to the ICCVAM agencies. NICEATM continues to work with the FETAX sponsor to assemble the necessary data, and it is anticipated that a Working Group will soon be organized.

There are several test methods with potential for future ICCVAM consideration. For example, as a result of the recent formal validation of the 3T3 NRU PT *in vitro* Phototoxicity test by the ECVAM Scientific Advisory Committee (ESAC), ICCVAM has formed a Phototoxicity Working Group to evaluate the ESAC report and recommend appropriate action to ICCVAM. In addition, there has been an Endocrine Disrupters Working Group formed to address anticipated submission of methods emerging from the Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC) established by the U.S. Environmental Protection Agency (EPA). Other methods with potential for ICCVAM review include Epiderm™ corrosivity assay, Human Corneal Epithelium (HCE-7) ocular irritancy assay, and transgenic mice models for carcinogenicity. ICCVAM continues to work closely with the Organization for Economic Cooperation and Development (OECD) on several issues primarily involving Guidance Documents on validation, humane endpoints in toxicity testing, and dermal absorption.

Bilateral acceptance

Finally, the notion of bilateral acceptance of test methods considered validated by the ICCVAM and ECVAM processes is achievable in the near future. The key elements are in place; we simply need to agree that the essential steps are present, and that the conclusions are based upon the time-tested expert peer review model. On behalf of FDA and the ICCVAM community, I congratulate ZEBET on its 10 year anniversary and wish continued success.

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Improving international co-operation between national organisations promoting the 3Rs

Summary

Co-operations between national centres on alternatives to animal experiments have two main advantages. The activities of a national centre will be placed in a broader perspective and the activities might be more effective due to the combined forces. Significant obstacles to come to co-operation are the lack of co-ordination and available finances. The formation of a European Consensus Platform (ECOPA), in which representatives of national Platforms participate, may overcome these obstacles.

Keywords: 3R, national centres, platforms, co-operation

Zusammenfassung: Verbesserung der internationalen Zusammenarbeit nationaler 3R-Organisationen

Aus der Zusammenarbeit mit nationalen Alternativforschungszentren ergeben sich zwei wichtige Vorteile. Erstens: Die Arbeit der Zentren steht auf einer breiteren Basis und zweitens: Durch gemeinsame Kräfte können sich synergetische Effekte ergeben. Bedeutende Hindernisse innerhalb der Zusammenarbeit beruhen auf mangelnder Koordination oder fehlenden Finanzen. Die Gründung einer European Consensus Platform *(ECOPA), in welcher auch nationale Vertreter teilnehmen, könnte diese Hindernisse aus dem Weg räumen.*

Situation in the Netherlands

In 1987, the Dutch Alternatives to Animal Experiments Platform (Platform) was initiated by the former Ministry of Welfare, Health and Culture to stimulate research leading to alternatives to animal experimentation and to co-ordinate necessary activities in this area. As the government acknowledged that the efforts concerning the replacement, reduction and refinement of animal experiments are the responsibility of the community as a whole, the different ministries and industry involved in animal experimentation, as well as animal welfare organisations were invited to participate in the Platform. Stimulation and co-ordination of the development and employment of alternative methods, which were the main objectives of the Platform, were achieved by financially supporting research projects.

In 1994, the Platform initiated the Netherlands Centre Alternatives to animal use (NCA). The main duty of the NCA was to support the Platform in its mission. The activities of the NCA involve disseminating information on alternatives, making an inventory of alternatives in the Netherlands, and stimulating and co-ordinating activities that contribute to the development and application of alternatives.

As part of a reorganisation of the responsible Ministry and the need for professio-

nal support from the Platform, the Minister for Public Health, Welfare and Sports has recently assigned the Health Research and Development Council (Dutch acronym: ZON) to carry out the funding and co-ordinating activities of the Platform. ZON is an independent organisation for programming and funding of research. An additional reason to assign ZON the co-ordination of activities in this field, is the explicit task and experience of ZON in promoting implementation of results of projects and research in daily practice. A multidisciplinary programme committee, consisting of key-persons representing four parties, viz. government, animal welfare, industry, and academia, will initiate, stimulate and monitor ZON's activities concerning alternatives research in the Netherlands. ZON and the NCA will closely co-operate to achieve their aims.

Situation in other countries

Like in the Netherlands, also in other countries initiatives concerning alternatives to animal experimentation have resulted in the founding of dedicated organisations. Some of the well-known organisations in Europe are the Fund for Replacement of Animals in Medical Experiments (FRAME, UK), Zentralstelle zur Erfassung und Bewertung von Ersatz- und Ergänzungsmethoden zu Tierversuchen (ZEBET, Germany), Stiftung zur Förderung der Erforschung von Ersatz- und Ergänzungsmethoden zur Einschränkung von Tierversuchen (set, Germany), Zentrum für Ersatz- und Ergänzungsmethoden zu Tierversuchen (zet, Austria), Fonds für versuchstierfreie Forschung, (FFVFF, Switzerland), and the recently established Belgian Platform for Alternative Methods (BPAM, Belgium). Also in other countries there is increasing attention to the concept of 3Rs (de Greeve and de Leeuw, 1997).

The origin and set up of the various organisations differ considerably. Some of the organisations are solely funded by charity money, the government funds others and some have mixed funds. Some organisations have their own research facilities, while others only fund research projects.

International initiatives

Not only at the national level initiatives have been taken to stimulate the three Rs. In 1986, the European Commission (EC) has published "Directive EC 86/609" concerning the protection of animals used for experimental and other scientific procedures (Anon, 1986). As a result of the political consensus that the development of alternative methods and their implementation should be supported, the Commission established the European Centre for the Validation of Alternative Methods (ECVAM).

There have also been initiatives that, in contrast to the national ones, are focused on specific aspects of the broad field of alternatives to animal experimentation. Examples well worth mentioning are the European Research Group on Alternatives to Toxicity Testing (ERGATT), the European Society for Toxicology In Vitro (ESTIV), the In Vitro Testing Industrial Platform (IVTIP), the European Network of Individuals and Campaigns for Humane Education (EuroNICHE) and the Advisory Group on Alternatives to Animal Testing in Immunobiologicals (AGAATI). These initiatives were started by individual researchers or research groups who recognised that international co-operation is essential in the field of alternatives to animal experimentation. However, in general, international co-operation is limited to specific research themes.

Need for co-operation

As international co-operation is recognised as imperative for scientific development, it is surprising that only a few formal international collaboration programmes have been set up between national centres for alternatives. This omission was already noticed at an ECVAM Workshop held in 1995. The participants of the workshop recommended that: "National, regional and international centres should be established to facilitate and promote research and the implementation of the three Rs through funding and education. These centres should be networked to facilitate co-ordination and information exchange". Furthermore, it was stated that: "Such organisations should consider joint efforts, as a means of improving quality, avoiding pitfalls, and sharing costs" (Balls et al., 1995).

As stated above, international co-operation will have advantageous effects on at least two levels. First, the activities of the individual centre will be situated in a broader perspective and secondly the activities might be more effective due to the international collaboration. For instance, most centres have insufficient financial resources to support all submitted projects. Exchange of information concerning proposed and ongoing projects will prevent the financial support of duplicate research or might initiate international collaboration. In the latter case, the result of the collaborative study might have a greater impact than in the case of an exclusively nationally supported project.

Most centres on alternatives act also as an information centre to scientists as well as to the general public. For both interest groups, information on the developments in other countries is essential as the need, use and elaboration of alternative methods does not end at the national borders.

Only a limited number of European countries have yet established a national centre or a comparable structure. These organisations have shown to be important in stimulating the research to alternative methods (de Greeve and de Leeuw, 1996; Anon., 1997). In Spain, the Czech Republic and Poland initiatives are currently set out for establishing a national funding organisation. International collaboration will prevent that these countries have to reinvent the wheel. The initiators might spend some valued time at existing national centres to learn how these are operating. A good example of such an initiative was demonstrated during the start-up phase of the recently instituted BPAM. Representatives of BPAM have visited other national centres and have adapted the strong points in their organisation to meet the Belgian needs and possibilities. Another example is the creation of the NCA database after consulting both FRAME and ZEBET. Their experiences and, among others, the NCA questions have been the reason for ECVAM to organise a workshop to start activities leading to more standardisation in databases (Janusch et al., 1997).

Causes of the lack of international co-operation

Although evident advantages can be identified when the national centres co-operate internationally, as was shown at a meeting organised by ECVAM in 1997, current practice shows that co-operation occurs only incidental. At the ECVAM meeting, representatives of European national

centres or equivalents, discussed distinguished items of mutual interest (*ATLA 25*, 492-493). However up-to-now no follow-up has been organised. Regardless the noble intentions, what could be the reason for the current lack of structural collaboration? To our opinion, two major obstacles can be identified. First of all, there is a lack of organising capacity. National centres have been set-up to organise and stimulate national developments. For that reason, no capacity has been designated for the time-consuming international collaboration. The second major reason might be the lack of funding. International collaboration is depending on information exchange and frequent communication. Although information can easily be exchanged via modern electronic pathways, regular meetings will enhance the efficacy of collaboration. These meetings and resulting actions are costly. National organisations often do not have sufficient resources to finance these activities.

Despite these significant hurdles, international co-operation should be endeavoured. In our opinion, co-operation will be successful when all actors in the field of alternatives to animal experimentation will take part. That means that representatives of government, industry (using experimental animals), academia, and animal welfare organisations should contribute. All four parties play a key-role in the feasibility of the use of alternative methods. The government will express their interest in relevant and reliable methods, e.g. for the assessment of public health or environmental risks. The government will strive to harmonised methods for transparent regulations and mutual acceptance of data. The participants of the industry will also be interested in harmonisation. Furthermore, their interest will be in determining areas in which a decrease in animal experimentation is possible. Academia will be interested in the incorporation of fundamental research needed in the development of alternative methods. Moreover, the contribution of the academic research might enforce the strength of the alternative methods by addition of knowledge on the mechanisms of action. Also, the input of the animal welfare organisations is important. These organisations have a great societal support, political influence and a critical opinion. Having the four parties participating will contribute to a continuing dialogue leading to information exchange, increased mutual understanding, and respect, decreased polarisation, and increased public and political awareness. We believe that an open attitude of all parties will lead to consensus on the mutual aims, e.g. reduction of animal experiments by promoting of the concept of Three Rs. Reaching international consensus on the priorities to be set will lead to higher efficiency. The limited resources will be invested in the most urgently needed research, of which the results will fit the regulators most wanted needs.

The above-mentioned approach has shown to be effective at the national level. In the last decade, the national platforms of Germany and The Netherlands were successful in supporting research to alternatives to animal experimentation (de Greeve and de Leeuw, 1996; Anon., 1997). In the funding organisations of these countries, ZEBET, set, Dutch Platform/ZON, all four parties participate in either the board or the programme committee. It is therefore desirable to establish a European organisation analogue to the national organisations in Germany, Belgium and The Netherlands.

International activities of platforms

We see great possibilities for the establishment of a European Consensus Platform (ECOPA). The strength of such an organisation is the participation of all interested parties. ECOPA could act as an expert advisory organ for national governments and European Commission and be consulted to set future targets and priorities at the European level. ECOPA's participants should be representatives of existing national Platforms.

An important off-spin of ECOPA could be the establishment of Platform-like organisations in countries where as yet no such organisations have been created.

Such a federation of co-operating national platforms could not only facilitate quality and information exchange, but also be responsible for the follow-up of some recommendations of the ECVAM workshop reports that require international co-ordination. For instance, ECVAM workshop report 25 on databases on alternative methods recommends: "A Central Reference Point (CRP) should be created to provide information on the contents of, and means of accessing, specialised databases and/or information services on alternatives. Preferably, the CRP should operate under auspices of an international organisation." Facilities like these can only be created by the combined efforts of national centres through the co-ordination of an international federation like the proposed ECOPA.

In addition, ECOPA could act as the organiser of regular meetings of national centres that are not structured like a platform, to further improve international collaboration.

In conclusion

We propose the creation of a European Consensus Platform (ECOPA) in which representatives of national Platforms participate. Like in the national Platforms, the participants of ECOPA represent national government and industry involved in animal experimentation, academia and animal welfare organisations.

One of the important activities of ECOPA could be the initiation of national Platforms in other European countries. ECOPA could act as an advisory organ to national governments and the EC, to propose future targets and priorities. Furthermore, ECOPA could serve as the co-ordinator of co-operation between national centres by organising annual meetings.

The Platforms in Germany, Belgium, and The Netherlands have agreed to explore the possibilities of ECOPA.

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Improving international co-operation between organisations promoting the 3Rs

Abstract

Co-operation between national centres involved in developing alternatives to laboratory animals is aimed at promoting the international implementation of the 3Rs (Reduction, Refinement and Replacement) to improve laboratory animal welfare and scientific research. Whilst there has been much formal collaboration between centres, scientists and regulators in numerous workshops and validation studies, less formal co-operation has been less extensive. This is due to insufficient time, lack of impetus and co-ordination. Such co-operation needs to be increased, however. This is because, although the principles of animal welfare and regulatory/GLP requirements are international, attitudes to animal experimentation, welfare standards for laboratory animals, as well as animal protection laws and guidelines vary. Improved international co-operation could facilitate agreement on unacceptable levels of animal suffering, international consensus on targets and time-limits for attaining specific goals in the 3Rs, and increased harmonisation of national legislation. Three possible approaches to improving international co-operation are considered: (a)- informal networking (via electronic mail); (b)- semi-formal networking (via the formation of an international platform for concerted action), and (c)- formal co-operation (via the creation of an independent international federation of national centres). It is concluded that the formation of a semi-formal platform of organisations is likely to be the best compromise in view of its cheapness and logistical simplicity. However, ways by which the formation and continued operation of such a platform can be ensured will need to be agreed between representatives of national centres.

Keywords: 3R, animal welfare, networking, co-operation, platform

Kurzfassung: Verbesserung der internationalen Zusammenarbeit von 3R-Organisationen

Die Zusammenarbeit nationaler Zentren, die sich der Entwicklung von Alternativmethoden widmen, bezweckt die Förderung der internationalen Anerkennung der 3R (reduction, refinement, replacement)*, um das Wohlergehen der Labortiere und die Forschung zu verbessern. Während es auf offizieller Ebene viel Zusammenarbeit zwischen Zentren, Wissenschaftlern und Zulassungsbehörden in zahlreichen Workshops und Validierungsstudien gegeben hat, kam die weniger formelle Zusammenarbeit seltener vor - ein Resultat aus Zeitmangel und einem Mangel an Initiative und Koordination. Indes muss diese Art der Zusammenarbeit mehr gepflegt werden, denn obschon die Prinzipien der Tierhaltung und die GLP* (Good Laboratory Practice)*-Richtlinien international sind, weisen die allgemeine Einstellung zu Tierversuchen, die Standards für die Haltung von Labortieren, die Tierschutzgesetze sowie die Richtlinien Unterschiede auf. Mit einer verbesserten internationalen Zusammenarbeit wäre es leichter möglich, sich auf einen unakzeptablen Leidensgrad von Versuchstieren zu einigen, einen internationalen Konsens für die Richtung und den Zeitrahmen zum Erreichen spezifischer 3R's-Ziele zu finden und eine grössere Harmonisierung nationaler Gesetzgebungen zu bewirken. Drei mögliche Ansätze zu einer solchen Verbesserung werden ins Auge gefasst: a) informelles Networking (durch elektronische Medien), b) teil-formelles Networking (durch die Schaffung eines internationalen Podiums für konzertierte Aktionen) und c) formelle Zusammenarbeit (durch die Schaffung eines unabhängigen internationalen Verbandes von nationalen Zentren). Es scheint, dass der zweite Vorschlag, die Schaffung eines teil-formellen Podiums, wegen der geringen Kosten und der logistischen Einfachheit den vorteilhaftesten Kompromiss darstellt. Allerdings braucht es für die Art und Weise, wie die Errichtung und die weiteren Operationen eines solchen Podiums gesichert werden können, die Zustimmung von Vertretern der nationalen Zentren.*

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Miroslav Cervinka and Zuzana Cervinková
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Co-operation between ZEBET and Charles University Faculty of Medicine

Abstract

By coincidence the co-operation between our faculty and ZEBET started in the same year when ZEBET was founded, and when the new era of our country begun. During these 10 years of co-operation very substantial progress towards modern attitudes in animal use was achieved in the Czech Republic. At the level of the whole country the most important achievement is that our national animal protection legislative is now fully compatible with the legislative in the European Union. At the level of our University the progress has been vitally connected with the help of three institutions: FRAME, ECVAM, and ZEBET; and with the positive attitudes of several people, namely Prof. M. Balls, Dr. R. Clothier, Prof. H. Spielmann and Dr. M. Liebsch. Co-operation between these institutions (people) was encouraged during TEMPUS Joint European Project "Alternatives to Experiments with Animals in Medical Education". Outcomes of these activities were dramatic decrease in the use of laboratory animals for educational purposes at our faculty, adoptions of strict regulations for the use of animals in the research, and widespread understanding of ideas of 3Rs. Very important role in this endeavour was played by several lectures of leading persons in this field, including Prof. W. Russell, presented at our faculty. These personal contacts help to change the attitudes among students and teachers towards the alternatives. Co-operation with the ZEBET is continuing even after completion of the TEMPUS project, recently mainly via established contacts and regular meetings organised by MEGAT. We are deeply obliged to our friends at ZEBET for their long lasting support, and we believe in close co-operation in the future. ZEBET vivat, crescat et floreat!

Keywords: 3R, replace, education, TEMPUS, medicine

Kurzfassung: Zusammenarbeit von ZEBET mit der medizinischen Fakultät der Karls Universität in Hradec Kralove Die Zusammenarbeit unserer Fakultät mit ZEBET begann zufälligerweise im gleichen Jahr, in dem ZEBET gegründet wurde und die neue Ära unseres Landes ihren Anfang nahm. Während dieser zehn Jahre hat sich in der tschechischen Republik die Einstellung gegenüber dem Verbrauch von Versuchstieren ganz wesentlich geändert. Auf gesamtstaatlicher Ebene wurde erreicht, dass die nationale Tierschutzgesetzgebung heute mit der entsprechenden EU-Gesetzgebung vollständig kompatibel ist. Auf Universitätsebene kam der Fortschritt vor allem durch die Mithilfe dreier Institutionen zustande: durch FRAME, ECVAM und ZEBET, sowie durch die positive Haltung von Persönlichkeiten wie Prof. M. Balls, Dr. R. Clothier, Prof. H. Spielmann und Dr. M. Liebsch. Die Zusammenarbeit mit diesen Institutionen und Persönlichkeiten begann während des TEMPUS Joint European Projects *"Alternativen zu Tierversuchen in der medizinischen Ausbildung". Ergebnisse dieser Aktivitäten waren ein dramatischer Rückgang bei Tierversuchen für Ausbildungszwecke in unserer Fakultät, die Annahme strikter Richtlinien für Tierversuche in der Forschung sowie ein zunehmendes Verständnis für die 3R Prinzipien. Eine wichtige Rolle spielten bei dieser Entwicklung auch Vorträge in unserer Fakultät, für die wir führende Fachleute gewinnen konnten, so auch Prof. W. Russell. Solche persönlichen Begegnungen sind für einen Gesinnungswandel zum Thema Alternativmethoden sowohl bei den Professoren wie auch bei der Studentenschaft sehr hilfreich. Die Zusammenarbeit mit ZEBET wird auch nach Abschluss des TEMPUS Projektes weitergeführt, in letzter Zeit hauptsächlich durch regelmässige Konferenzen, die von MEGAT organisiert werden. Wir sind unseren Freunden bei ZEBET für ihre langdauernde Unterstützung zutiefst dankbar und vertrauen auf eine enge Zusammenarbeit auch in der Zukunft. ZEBET lebe, wachse und gedeihe!*

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Regulatoray Toxicology: Validation of *in vitro* Tests and Test Strategies at the BgVV

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Alternatives to the Draize eye test: Current status of validation and acceptance in Europe

Abstract
The use of the Draize eye test for the safety assessment is described for chemicals that are severely irritant, moderately or even non-irritant to the eye and also for pharmaceuticals that are applied to the eye in ophthalmology. The current approaches to develop and validate in vitro *alternatives to the Draize eye test will be outlined. Despite progress in this particular field of* in vitro *toxicology, non of the alternative tests has been accepted so far at the international level by regulatory agencies or the OECD. In Germany, the HET-CAM test on the embryonated chicken allantois membrane in combination with a cytotoxicity assay is accepted by the competent authorities to classify severely eye irritating materials. This type of classification is accepted by all of the EU Member states, which also accept* in vitro *classification of severely irritating materials, if the data have been obtained in the BCOP test on the isolated bovine cornea and the isolated chicken eye (ICE) test - both from slaughter house material - and also in the isolated rabbit eye (IRE) test. Chemicals providing a negative result in the four* in vitro *tests still have to be tested in the Draize eye test in rabbits in order to prove that they are non-irritating to the eye.*
Today, in EU member states the eye irritation potential of finished cosmetic products, which are in general not irritating to the eye, is assessed only by in vitro *alternatives to the Draize eye tests. The* in vitro *tests employed in laboratories of the cosmetic industry are "in house tests", which have never undergone a formal validation procedure. However, validation studies of* in vitro *tests to assess the eye irritation potential of new chemicals, which are used in cosmetic formulations, have so far not been successful except for an* in vitro *test using an artificial skin model, which is not available commercially any more. According to experts of both the EU SCCNFP (the Scientific Committee on Cosmetology and Non-Food Products) and COLIPA (the European Cosmetic, Toiletry and Perfumery Association), it will take several years until a validated* in vitro *test for assessing the eye irritation potential of cosmetic ingredients will have been successfully validated and accepted for regulatory purposes.*
Taking the public concern on the one hand and the slow progress on the other hand into consideration, the EU validation centre ECVAM in Ispra, Italy, and COLIPA have held several joint workshops in 1997-1998 to agree on the best way forward. As a result, several international validation projects are under way to replace the Draize eye test for the evaluation of mildly and non-irritating chemicals in the very near future. They are funded both by the EU and the European cosmetic industry.

Keywords: 3R, replace, Draize eye test, validation, Het-CAM, BCOP bovine cornea, ICE isolated chicken eye test, IRE rabbit eye test

Kurzfassung: Alternativmethoden zum Draize Test am Kaninchenauge: Ergebnisse von Validierungsstudien und behördliche Anerkennung in der EU
Der Draize Test am Kaninchenauge wird zur sicherheitstoxikologischen Bewertung chemischer Stoffe eingesetzt, um zu bestimmen, ob sie stark, gering oder nicht augenreizend wirken, und außerdem in der Augenheilkunde, um die Unbedenklichkeit von Arzneizubereitungen in der Augenheilkunde zu bestimmen. Es wird ein Überblick über abgeschlossene Validierungsstudien von Ersatzmethoden zum Draize Test gegeben. Trotz deutlicher Fortschritte bei der Entwicklung von Ersatzmethoden wurde bisher noch keine dieser Alternativmethoden uneingeschränkt für behördliche Zwecke international anerkannt. In Deutschland wird zur Bestimmung stark augenreizender Eigenschaften der HET-CAM Test am bebrüteten Hühnerei in Kombination mit einem Zytotoxizitätstest von den zuständigen Behörden als Ersatzmethode anerkannt. Die Einstufung stark reizender Stoffe, die mit diesem in vitro *Test erhoben wurde, wird von den Behörden aller Mitgliedsstaaten der EU akzeptiert. In gleicher Weise wird die Einstufung stark augenreizender Stoffe in der EU mit dem BCOP-Test an der isolierten Rinder-Cornea aus Schlachthofmaterial anerkannt, sowie im Test mit isolierten Augen von Hühnern (ICE Test) und Kaninchen (IRE-Test), die ebenfalls an Schlachthofmaterial durchgeführt werden. Stoffe, die mit einem der genannten* in vitro *Tests zu einem negativen Ergebnis führen, müssen zum Nachweis der Unbedenklichkeit noch im Draize Test an einem Kaninchen geprüft werden. Auf diese Weise wird die Testung stark augenreizender Stoffe im Kaninchen in der EU vermieden, und die Zahl der Draize Tests, die mit augenreizenden Stoffen durchgeführt werden, konnte in Europa erheblich reduziert werden.*
Kosmetische Fertigprodukte, die üblicherweise wenig augenreizend wirken, werden heute in der EU ausschließlich mit in vitro *Tests auf ihre augenreizenden Eigenschaften geprüft. Tests, die dazu in den Laboratorien der Kosmetikindustrie angewandt werden, sind überwiegend firmenintern entwickelte Tests, die niemals unter blinden Bedingungen experimentell validiert wurden, wie das heute mit neuen* in vitro

*Toxizitätstests erforderlich ist. In umfangreichen Validierungsstudien von Ersatzmethoden zum Draize Test, die unter Federführung des Europäischen Verbandes der Kosmetikindustrie COLIPA durchgeführt wurden, erwies sich keiner der Tests als ausreichend reproduzierbar und prädiktiv mit Ausnahme eines Tests, bei dem ein künstliches, menschliches Hautmodell eingesetzt wird, das der menschlichen Cornea ähnlich ist. Dieser kommerziell entwickelte und vertriebene Test ist leider nicht mehr auf dem Markt. Nach Ansicht der in der EU für die Sicherheit von Kosmetika zuständigen Kommission SCCNFP (*Scientific Committee on Cosmetology and Non-Food-Products*) und nach Ansicht von COLIPA wird es noch mehrere Jahre dauern, bis ein experimentell validierter* in vitro *Test zur Prüfung chemischer Stoffe zur Verfügung stehen wird, zu denen auch Kosmetikinhaltsstoffe gehören.*

Unter Berücksichtigung der hochgesteckten Erwartungen der Bürger in der EU einerseits und der recht schleppenden Fortschritte bei der Entwicklung von Alternativmethoden zum Draize Test andererseits, haben das EU Validierungszentrum ECVAM und COLIPA in den Jahren 1997-1998 gemeinsam mehrere Workshops abgehalten, um zu einer möglichst raschen Lösung zu kommen. Als Ergebnis führen ECVAM und COLIPA seit 1998 mehrere abgestimmte Forschungsprojekte durch, um den Draize Test am Kaninchenauge auch bei der Prüfung auf geringe augenreizende Eigenschaften bzw. das Fehlen von augenreizendem Potential möglichst bald zu ersetzen. Diese Studien werden von der EU, von ECVAM und von der europäischen Kosmetikindustrie finanziert.

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Acute toxic class methods: Alternatives to LD/LC$_{50}$ tests
This contribution is published as an article in ALTEX 3/99 (129-134). ALTEX is mourning for Wolfgang Diener, who died a few weeks after the presentation of this manuscript at ZEBET's anniversary.

Julia H. Fentem
Unilever Research,UK-Sharnbrook:
Validation of *in vitro* tests for skin corrosivity
This contribution is presented as a short communication in ALTEX 3/99 (150-153).

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Validation of *in vitro* phototoxicity tests: current status and future perspectives

Abstract:
Since no standardised international guideline for the testing of chemicals for phototoxic potential had been accepted for regulatory purposes, in 1991, on initiative of ZEBET, the European Commission (EC), represented initially by the Directorate General XI (DG XI) and later by ECVAM (the European Centre for the Validation of Alternative Methods) and COLIPA (the European Cosmetic, Toiletry and Perfumery Association), agreed to establish a joint EU/COLIPA programme on the development and validation of in vitro *phototoxicity tests. The first phase (phase I, 1992-93) was designed as a prevalidation study, to identify promising* in vitro *tests for a formal validation trial under blind conditions.*
In phase II (1994-95), the formal validation study, the most promising in vitro *phototoxicity tests were assessed with 30 carefully selected test chemicals in a blind trial. The 3T3 mouse fibroblast neutral red uptake phototoxicity test (3T3NRU-PT) was validated as a core test in nine laboratories, since it provided the best results in phase I of the study. The other* in vitro *tests were assessed in a smaller number of laboratories. Results obtained with the 3T3NRU-PT were reproducible and the correlation between* in vitro *and* in vivo *data was very high.*
In 1996, the Scientific Committee of Cosmetology (SCC) of DG XXIV of the EC asked ECVAM to test UV filter chemicals from the 1995 edition of Annex VII of Directive 76/768/EEC *in a blind trial in the 3T3 NRU PT test. A COLIPA committee selected eight UV filter chemicals, for which the absence of phototoxic potential was backed by* in vivo *data (animals and humans) of sufficient quality. To balance the number of positive and negative chemicals, 10 phototoxic and 10 non-phototoxic chemicals were tested under blind conditions in four laboratories. Again, the results obtained were highly reproducible, and the correlation between* in vitro *and* in vivo *data was almost perfect. All phototoxic test chemicals provided a positive result at concentrations of 1 mg/ml, while nine of the ten non-phototoxic chemicals gave clear negative results, even at the highest test concentrations. An analysis of the impact of exposure concentrations on the test performance revealed that the optimum concentration range in the 3T3NRU-PT test is 0.1-100 mg/ml, and*

that false positive results may be obtained only at chemical concentrations exceeding 100 mg/ml.
Independent evaluation of the studies by the ECVAM Scientific Advisory Committee (ESAC) led to endorsements qualifying the 3T3NRU PT (1) as scientifically validated test ready to be considered for regulatory acceptance *in October 1997, and (2)* as applicable to UV filter chemicals *in March 1998. The endorsements were followed by statements on the acceptance of the 3T3NRU-PT by DG XI and DG III, and finally by the SCCNFP of DG XXIV in November 1998. A draft OECD Test Guideline for* „In Vitro *Phototoxicity Testing", based on the standard protocol of the 3T3NRU-PT, has been submitted by the DG XI to the OECD test guidelines programme in September of 1998.*
With the support of initially DG XI and later ECVAM, ZEBET had developed in vitro *phototoxicity tests with 3D reconstructed human skin models (1992: LSE from Organogenesis, and 1993: Skin² from ATS) of which the latter performed very well when tested under blind conditions in phase II of the EU/COLIPA trial. After production stop of Skin², in 1997-98 in a prevalidation study funded by ECVAM, we have successfully adopted the robust Skin² test protocol to the human epidermis model EpiDerm™ (MatTek), which showed excellent reproducibility and predictivity in a final blind trial performed with Beiersdorf and Procter & Gamble. The use of this test for safety/potency testing, as well as other areas of phototoxicology (photogenotoxicity, photocarcinogenicity, and photoallergy) will be evaluated in the ECVAM Workshop* „In Vitro *Phototoxicity Testing 2" following this Symposium.*

Keywords: 3R, replace, phototoxicity, testing strategy, validation, regulatory acceptance, UV-filter, human skin model, EpiDerm™

Kurzfassung: Validierung von in vitro *Phototoxizitätstests: Gegenwärtiger Stand und Aussichten*
Da es keine international behördlich anerkannte Prüfvorschrift für die Testung des phototoxischen Potentials von Stoffen gab, hat ZEBET 1991 ein Verbundprojekt zwischen der EU (zunächst repräsentiert durch die DG XI und später durch ECVAM) und dem Europäischen Verband der Kosmetik-, Körperpflegemittel- und Parfümhersteller (COLIPA) zur Entwicklung und Validierung von in vitro *Phototoxizitätstests initiiert. Die erste Phase (1992-93) wurde als Prävalidierung zur Identifikation erfolgversprechender* in vitro *Testprotokolle durchgeführt.*
In der Phase II, einer formalen Validierungsstudie, wurden 30 sorgfältig ausgewählte Testchemikalien mit den erfolversprechendsten in vitro *Phototoxizitätstests unter blinden Bedingungen geprüft. Der 3T3-Neutralrot-Aufnahme-Phototoxizitätstest (3T3NRU-PT) mit Fibroblasten der Maus wurde als wichtigster „Pflichttest" in neuen Laboratorien geprüft, da er in Phase I der Studie die besten Resultate bei der Vorhersage des phototoxischen Potentials von Stoffen erbracht hatte, während die anderen Phototoxizitätstests in einer kleineren Zahl von Laboratorien geprüft wurden. Wie schon in Phase I, waren die mit dem 3T3NRU-PT erzielten Ergebnisse gut reproduzierbar und die Korrelation zwischen den* in vitro *Ergebnissen und den phototoxischen Eigenschaften* in vivo *war sehr hoch.*
Im Jahr 1996 hat dann das „Scientific Committee on Cosmetology (SCC)" *der für Kosmetika zuständigen DG XXIV der EU den Wunsch geäußert, daß mit dem inzwischen validierten 3T3NRU-PT noch eine weitere Studie unter blinden Bedingungen durchgeführt wird, in der UV-Filterstoffe aus Annex VII (Positivliste) der Kosmetikrichtlinie 76/768/EEC getestet werden. Eine COLIPA Expertengruppe hat daraufhin acht UV-Filterstoffe auswählen können, für die die Abwesenheit phototoxischer Eigenschaften hinreichend gut durch* in vivo *Studien an Tieren und am Menschen belegt war. Um die geplante Blindstudie hinsichtlich der phototoxischen Eigenschaften der Teststoffe zu balancieren, wurden weitere Stoffe ergänzt, so daß insgesamt 10 phototoxische und 10 nicht phototoxische Stoffe in vier Laboratorien der Industrie blind getestet wurden. Abermals waren die erzielten Ergebnisse hoch reproduzierbar und die* in vivo/in vitro *Korrelationen nahezu perfekt. Darüber hinaus wurde durch ein sorgfältiges Design der Studie sichergestellt, daß alle Testsubstanzen nur innerhalb ihrer Löslichkeitsgrenzen geprüft wurden. Dabei ergab sich, daß alle geprüften Phototoxine ihre toxische Eigenschaft bereits bei 1 mg/mL zeigen, während die nicht phototoxischen Stoffe bis zu einer Konzentration von 100 mg/mL ein richtig negatives Ergebnis im 3T3NRU-PT aufweisen und nur oberhalb dieser hohen Konzentration vereinzelt Artefakte auftraten, die zu einer falsch positiven Klassifizierung führten.*
Die unabhängige Bewertung der Ergebnisse der Validierungsstudie und der UV-Filterstudie durch das „ECVAM Scientific Advisory Committee (ESAC)" *führte zu folgenden Empfehlungen: „Der Test ist ...(1) wissenschaftlich validiert und wird zur behördlichen Anerkennung empfohlen (2) anwendbar für die Prüfung von UV-Filterstoffen." Diesen positiven Würdigungen durch ESAC im Oktober 1997 und im März 1998 folgten offizielle Anerkennungen des Tests durch die DG XI (Verbraucherschutz, Testmethoden), DG III (Industrie) und schließlich auch durch das* „Scientific Committee on Cosmetology and Non Food Products (SCCNFP)" *der DG XXIV im November 1998. Der Entwurf einer neuen OECD Prüfvorschrift* „In Vitro *3T3 NRU Phototoxicity Test" wurde im September 1998 durch die DG XI bei der OECD eingereicht.*
Mit Unterstützung durch die DGXI und später durch ECVAM hat ZEBET darüber hinaus in vitro *Phototoxizitätstests mit dreidimensionalen Modellen rekonstruierter menschlicher Haut entwickelt: Dies waren 1992* „Living Skin Equivalent (LSE)" *der Firma Organogenesis, USA und ab 1993 „Skin² „ der Firma Advanced Tissue Sciences, USA. Der letztere Test war in der Phase II des EU/COLIPA Projektes mit sehr guten Ergebnissen unter blinden Bedingungen getestet worden. Nach dem überraschenden Produktionsstop von Skin² haben wir daher in einer von ECVAM geförderten Prävalidierungsstudie erfolgreich das robuste Skin² Testprotokoll auf das Modell EpiDerm™ (rekonstruierte menschliche Epidermis) angewendet und konnten nach geringfügiger Anpassung in drei Laboratorien (Procter & Gamble, Beiersdorf und*

*ZEBET) die hervorragende Reproduzierbarkeit und Prädiktivität unter blinden Testbedingungen zeigen. Über den möglichen Einsatz dieses Tests (der sich aufgrund der vorhandenen Hautbarriere als Sicherheitstest oder zur Abschätzung der phototoxischen Potenz ausbauen ließe) wird im Rahmen des im Anschluß an dieses Symposium stattfindenden ECVAM Workshops „*In Vitro *Phototoxicity Testing 2" diskutiert. Der Workshop wird weiterhin als spezielle Bereiche der Phototoxizität, die Photogenotoxizität, die Photokarzinogenität und die Photoallergie behandeln.*

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Embryotoxicity screening using ES cells *in vitro*: Correlation to *in vivo* teratogenicity

Abstract

Blastocyst-derived totipotent embryonic stem (ES) cells of the mouse can be induced to differentiate in culture into a variety of cell types, including cardiac muscle cells. The embryonic stem cell test (EST) that makes use of the differentiation of ES cells into cardiomyocytes in a standardised in vitro *model, was developed to offer an alternative method to comprehensive* in vivo *studies in reproductive toxicology about toxic effects of chemicals. ES cells of the mouse cell line D3 are investigated for their preserved capability to differentiate following drug exposure, and both ES cells and differentiated fibroblast cells of the mouse cell line 3T3 are comparatively analysed for effects on viability. The following endpoints are used to classify the embryotoxic potential of chemicals into three classes of* in vitro *embryotoxicity (non, weak or strong embryotoxic). These endpoints are: (i) the inhibition of differentiation of ES cells into cardiomyocytes after 10 days of treatment and the decrease of viability (cytotoxicity) of (ii) 3T3 cells and (iii) ES cells after 10 days of treatment, determined by an MTT test. 50% inhibition concentrations for differentiation (ID_{50}) and cytotoxicity ($IC_{50}D3$ and $IC_{50}3T3$) are calculated from concentration-response curves. Applying linear analysis of discriminance, a biostatistical prediction model (PM) was developed. This procedure identified three variables, the $lg(IC_{50}D3)$, the $lg(IC_{50}3T3)$ and the relative distance between $IC_{50}3T3$ and ID_{50}, that improved the separation of the three classes of embryotoxicity compared to the prediction model that was originally proposed after test development (Spielmann et al., 1997). Unlike the original PM, the improved PM incorporates as one variable the relative distance between $IC_{50}3T3$ and ID_{50}, instead of the ratio $ID_{50}/IC_{50}D3$, that was used previously.*

Keywords: 3R, in vitro differentiation, embryonic stem cell test (EST), cardiomyocytes, cytotoxicity, embryotoxicity, prediction model (PM)

Kurzfassung: Die Prüfung embryotoxischer Substanzen mit embryonalen Stammzellen in vitro*: Korrelation zur* in vivo *Teratogenität*

Aus der inneren Zellmasse von Maus-Blastozysten gewonnene pluripotente embryonale Stammzellen (ES-Zellen) der Maus können unter definierten Kulturbedingungen in vitro *in eine Reihe verschiedener Zelltypen differenzieren. Der bei ZEBET entwickelte Embryonale Stammzelltest (EST) nutzt in einem standardisierten* in vitro *Modell die Differenzierung von ES-Zellen in spontan kontrahierende Herzmuskelzellen (Cardiomyozyten). Der EST wurde als Alternativmethode zu Tierversuchen in der Reproduktionstoxikologie entwickelt, um embryotoxische/teratogene Effekte von Chemikalien zu identifizieren. Dabei werden ES-Zellen der Maus-Zellinie D3* in vitro *auf ihre Fähigkeit zur Differenzierung unter Einwirkung von Testsubstanzen untersucht. Dies wird mit dem Effekt von Testsubstanzen auf die Vitalität von ES-Zellen und differenzierten Fibroblasten der Maus-Zellinie 3T3 verglichen. Die folgenden Endpunkte werden zur Klassifizierung des embryotoxischen Potentials von Chemikalien in drei Klassen der* in vitro *Embryotoxizität (nicht, schwach oder stark embryotoxisch) eingesetzt: (i) die Hemmung der Differenzierung von ES-Zellen in Cardiomyozyten nach einer Behandlungsdauer von 10 Tagen, (ii) die Verminderung der Vitalität (Zytotoxizität) von 3T3 Fibroblasten und (iii) die Verminderung der Vitalität von ES-Zellen nach einer Behandlungsdauer von 10 Tagen. Zytotoxische Effekte werden in einem Standard-Vitalitätstest, dem MTT-Test, untersucht. 50% Hemmkonzentrationen für die Differenzierung (ID_{50}) und für die Zytotoxizität ($IC_{50}D3$ und $IC_{50}3T3$) werden anhand von Konzentrations-Wirkungs-Kurven bestimmt.*

Durch die Anwendung linearer Diskriminanzanalysen konnte ein biostatistisches Prädiktionsmodell entwickelt werden. Dieses Verfahren identifizierte drei sog. "Variablen": der Logarithmus der $IC_{50}D3$, der Logarithmus der $IC_{50}3T3$ und die relative Distanz zwischen $IC_{50}3T3$ und ID_{50}, die zu einer sehr guten Trennung in die drei Klassen der Embryotoxizität führten. Im Vergleich zu dem ursprünglich nach der Testentwicklung vorgeschlagenen Prädiktionsmodell (Spielmann et al., 1997), das als eine von drei Variablen den Quotienten $ID_{50}/IC_{50}D3$ benutzt, führt die "neue" Variable, die relative Distanz zwischen $IC_{50}3T3$ und ID_{50}, zu einer deutlich verbesserten Vorhersage der Embryotoxizität. Damit wurden 93% aller Einzelexperimente der "Lernstichprobe" korrekt vorhergesagt.

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Introduction of SAR considerations and specific *in vitro* tests into skin and eye irritation/corrosion testing strategies

Abstract:
For the minimisation of animal testing within the notification procedure of new chemical substances in the European Union the development of stepwise assessment procedures including structure-activity-considerations, alternative methods like in vitro *tests and computerised structure-activity-relationships (SAR-models) are needed and necessary.*
In order to find these testing strategies supporting the assessment of skin and eye irritant/corrosive substance properties and the subsequent correct allocation of respective EU risk-phrases, test protocols submitted within the notification procedure for new chemicals were evaluated. In a first step relationships between physico-chemical properties and skin and eye irritation/corrosion caused by a chemical substance were examined. Based on the results of this evaluation a computerised system for the prediction of local irritancy or corrosivity on skin and/or eyes by means of structure-activity considerations was developed. This system supports - based upon theoretical structure activity relationships considerations nominated by the system - the decision whether reliable prediction of local irritancy/ corrosivity to skin and/or eyes can be made
1) by means of SAR considerations only, no further testing
2) by means of a combination of SAR considerations and results of a specific alternative test (e.g., one of the recently validated alternative methods for the assessment of skin corrosivity) or
3) only by means of an animal test (because the substance will produce no or only marginal local irritant effects on skin and/or eyes. At present, EU regulations do not accept alternative methods for the assessment of slight irritant properties).

Keywords: 3R, testing strategies, SAR considerations, decision support systems, skin irritation/corrosion, eye irritation

Kurzfassung: Einführung von Strukturwirkungsüberlegungen und spezifischen in vitro*-Methoden in Teststrategien zur Bewertung lokaler Reizwirkungen*
Um die Anzahl der für die Anmeldung neuer Chemikalien in der Europäischen Union noch zu fordernden Tierversuche auf ein Minimum zu reduzieren, werden sequentielle Bewertungsstrategien entwickelt. Für derartige Bewertungsstrategien werden Strukturwirkungsüberlegungen, Alternativmethoden wie beispielsweise in vitro *Tests und computergestützte Strukturaktivitätsmodelle (SAR-Modelle) benötigt.*
Zum Zweck der Entwicklung von Teststrategien zur toxikologischen Bewertung von Haut- und Augenreizwirkungen chemischer Stoffe und deren Klassifizierung (R-Sätze der EU als Hinweise auf besondere Gefahren) wurden die Prüfprotokolle ausgewertet, die den Behörden im Rahmen der Anmeldung von Chemikalien einzureichen sind. In einem ersten Schritt wurden Zusammenhänge zwischen physikalisch-chemischen Stoffeigenschaften und Ätz- und Reizwirkungen an Haut und Augen untersucht. Die dabei erkannten Gesetzmäßigkeiten wurden zum Aufbau eines computergestützten Entscheidungsfindungssystems herangezogen. Dieses System gibt - auf der Basis von theoretischen Betrachtungen über Strukturwirkungszusammenhänge, die das System konkret benennt - Entscheidungshilfen, ob zur Vorhersage lokaler Reiz- oder Ätzwirkungen an Haut und/oder Augen
1) allein theoretische SAR-Überlegungen genügen, weitergehende Tests sind in diesem Fall nicht erforderlich
2) eine Zusammenschau aus SAR-Betrachtungen und den Ergebnissen spezieller Alternativmethoden ausreicht (z.B. Testergebnisse, die mit den kürzlich validierten Methoden zur Bewertung hautätzender Stoffwirkungen erhalten wurden) oder
3) Tierversuche durchgeführt werden müssen (weil der Stoff vermutlich keine oder nur sehr schwache Reizwirkungen an Haut und/oder Augen zeigt. Die gegenwärtigen EU-Vorschriften gestatten aus Vorsorgegründen nicht, die Unbedenklichkeit eines Stoffes mit Hilfe von Alternativmethoden nachzuweisen).

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Institutions Funding Research According to the 3Rs

Michael Balls
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The funding of research on the 3Rs in the EU

Abstract
In a number of European countries, notably Germany, The Netherlands, Sweden and the UK, there has been a significant commitment to research designed to achieve one or more of the 3Rs. This work has been funded by industry, by government and by the animal welfare movement. The cosmetic industry, ZEBET in Germany and the Dutch Platform in The Netherlands, and FRAME in the UK, deserve particular mention. The European Commission also plays a major role, in two main ways. Firstly, research is supported via DGXII, particularly through programmes such as BRIDGE, BIOTECH and BIOMED in the Third and Fourth Framework Programmes (1991-1998). In the Fifth Framework Programme (1999-2002), major support for international collaborative studies on the development of replacement alternative test methods will be provided as parts of programmes concerned with The Cell Factory (novel in vitro *testing as alternatives to animal testing) and Environment and Health (improvement of predictive toxicity testing, with emphasis on* in vitro *test systems and alternative screening and testing protocols). The second kind of funding is by competitive contracts for specific studies required by various Services of the Commission, including, for example, prevalidation and validation studies conducted for ECVAM.*

Keywords: 3R, EU funding, framework programmes, The Cell Factory, Environment and Health, validation studies, ECVAM

Kurzfassugng: Die Finanzierung der 3R-Forschung in der EU

Verschiedene europäische Staaten, vornehmlich Deutschland, Holland, Schweden und das Vereinigte Königreich haben für die Forschung, die eines oder mehrere Ziele der 3R anstrebt, grossen Einsatz geleistet. Finanziert wurden die Anstrengungen durch die Industrie, mit öffentlichen Geldern und durch Tierschutzorganisationen. Die Kosmetikindustrie und ZEBET in Deutschland, die Dutch Platform in Holland und FRAME im Vereinigten Königreich verdienen besondere Erwähnung. Eine Hauptrolle spielt die Europäische Kommission, und zwar in zweierlei Hinsicht: Einmal wurde die Forschung durch die DGXII, im besonderen durch die Programme BRIDGE, BIOTECH und BIOMED im dritten und vierten Rahmenprogramm (1991-1998) unterstützt. Im fünften Rahmenprogramm (1999-2002) wird die Hauptförderung für international koordinierte Studien zur Entwicklung von alternativen Testmethoden als Teil der The Cell Factory-Programme bereitgestellt, sowie bei Environment and Health, d.h. zu Verbesserungen in der Risikoabschätzung der Toxizität mit Schwerpunkten auf in vitro *Testsystemen und für alternative Screeningverfahren und Testprotokolle. Die zweite Möglichkeit der Beschaffung von Fördermitteln ist die Ausschreibung von Forschungswettbewerben für spezielle Studien, die von verschiedenen Organen der Kommission angefordert werden, so z.B. auch Prävalidierungs- und Validierungsstudien für ECVAM.*

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Funding for activities involving the 3Rs in the United States

Summary
Not unlike the situation in Europe, most of the U.S. funding for research on the 3Rs - methods that refine, reduce, or replace the use of laboratory animals - comes from four sources: a) the national Government, generally in the form of National Institutes of Health grants or contracts, b) industry (including their associated trade associations), c) the animal protection community and alternative organizations, and d) companies which are directly involved in developing commercial products for alternative applications. The difference lies with how the practical development and maturation of alternative methods are funded. Unlike Europe, U.S. Government funds are rarely targeted towards research with the specific purpose of advancing the 3Rs; rather the money supports basic biochemical and in vitro *research which eventually results in gains for the 3Rs by increasing the general scientific knowledge. There is virtually no U.S. Government funding for optimization of methods, or prevalidation and validation studies. These latter, extremely important, activities have historically received their funding from industry and developers of commercial products*

for the alternative fields. Recently the animal welfare community has begun lending increased support to these activities, and this will hopefully allow increased validation efforts in the U.S. However, for real progress to be made, the U.S. Government must begin to provide more funding for studies which move 3Rs methods further along the path to regulatory acceptance.

Zusammenfassung: Die Finanzierung von 3R-Aktivitäten in den U.S.A.
Ähnlich wie in Europa fliessen auch in den U.S.A. die meisten Gelder für die 3R-Forschung (reduce, replace, refine) aus vier Quellen: a) von der Regierung, meist in Form von NIH-Beiträgen oder Verträgen, b) von Industriefirmen (inklusive deren Handelsgesellschaften), c) aus der Tierschutzbewegung und von Organisationen zur Förderung von Alternativmethoden und d) von Firmen, die direkt mit der Entwicklung kommerzieller Produkte für Alternativmethoden befasst sind. Die Unterschiede liegen in der Art und Weise, in der die Entwicklung von Alternativmethoden bis hin zur Praxisreife gefördert wird. Im Gegensatz zu Europa sind die Förderbeiträge der U.S. Regierung im allgemeinen nicht speziell auf die 3R-Forschung ausgerichtet; es ist eher so, dass die Geldmittel allgemein für biochemische und in vitro *Forschungsprojekte bestimmt sind, aus denen dann eventuell 3R relevante Ergebnisse resultieren. Letztlich gibt es keine Förderung der U.S.-Regierung für die Optimierung von Methoden oder Prävaliderungs- und Validierungsstudien. Gerade die letzteren, extrem wichtigen Aktivitäten wurden, historisch gesehen, immer von der Industrie und kommerziellen Anbietern von Alternativmethoden gefördert. In jüngster Zeit haben vor allem auch Tierschutzorganisationen sehr intensiv diese Entwicklung gefördert. Dies erlaubt die Hoffnung, dass in den U.S.A. verstärkt Validierungsbemühungen erkennbar werden. Um jedoch echte Fortschritte zu erzielen, müsste die U.S.-Regierung vermehrt Studien fördern, die gezielt 3R-Methoden auf den Weg zur behördlichen Akzeptanz bringen.*

Keywords: 3R, U.S. research funding, alternative methods, validation studies, industry, animal welfare community

U.S. government funding

The U.S. Government (mainly through the National Institutes of Health, NIH) funds hundreds of millions of dollars in basic research in biochemistry, tissue culture, pharmacology, general toxicology, etc. Growth of each of these disciplines is of tremendous value to the development of alternative methods. Therefore funding of these areas eventually results in gains for the 3Rs. It is rare, however, to find funds that are specifically designated for 3R's activities, especially those activities such as pre-validation and validation studies which move an immature alternative method forward so that eventually it is successfully utilised by industry.

There are a few exceptions where research that advances the 3Rs is specifically mentioned in solicitations for funding. One is in the Small Business Innovative Research Grants (SBIR) program. Here various program departments can suggest specific areas in which they would like to receive proposals. It is encouraging that both the Office of Research Resources and the National Institute of Environmental Health Sciences (NIEHS) both mention their desire to fund some projects which reduce, refine or replace the use of animals in laboratory experiments.

In the SBIR program funds are given to small businesses (not-for-profit entities are excluded from being the principle recipient of the funding, although they can receive subcontracted funds) to aid in the development of new products, e.g. a new tissue model. Funding is quite significant; up to $100,000 for the first phase (generally one-year) and up to $750,000 for a second phase (generally two years). Again, this type of funding supports methods development. No provisions are made for support of pre-validation or validation studies.

Another way the Government provides financial support for alternatives is through funding of ICCVAM, the Interagency Coordinating Committee on the Validation of Alternative Methods. This group is charged with facilitating the acceptance of alternative methods by U.S. regulatory agencies. However, unlike ECVAM in the Europe, no funds have been appropriated ICCVAM to conduct prevalidation or validation studies. Thus there is little or no oversight of the entire process of alternative method developments. In such a situation, even promising methods can become „lost" when there is no central authority (as in Europe) to shepherd their progress. While at present ICCVAM does not have funds to directly support methods development, it can fund workshops on new assays that can serve to accelerate interest in, and development of, alternative methods.

U.S. industry funding

Industry (both individual companies and trade associations) has been one of the most significant sources of U.S. funding for 3Rs activities. Driven by the pressure to utilise fewer animals, while at the same time needing increasingly more detailed safety and efficacy information, industry has directed hundreds of millions of dollars into the development of alternative methods. Generally these funds have supported replacement and reduction activities, with fewer funds going to refinement.

Industry provides support not only for internal research that is directly beneficial to their product lines, but also for research conducted by external investigators. Funding can quickly flow through a contract mechanism or can be given through the more protracted process of a competitive funding program. Major consumer products companies such as The Procter & Gamble Company, The Gillette Company, and Colgate-Palmolive Company have all conducted programs of this type. Although most industry money supports projects that are likely to directly benefit the company, a number also support general alternative research that can benefit the entire field.

The amount of money spent by industry on alternative research is not trivial. Both The Gillette Company and Colgate-Palmolive Company spent over $1.3 mil-

lion dollars in 1998. The Procter and Gamble Company estimates that it spent over $5 million dollars last year on external funding. In addition almost all companies contacted indicated that their alternatives expenditures has continued to increase over the last five years.

Alternatives organisations

Industry, as well as animal protection organisations and other stakeholders, also indirectly fund 3R's research through contributions to alternatives organisations such as CAAT (Center for Alternatives to Animal Testing at Johns Hopkins University). Since its founding in 1982, CAAT has supported 3R's research with 245 grants totalling ~$4.5M. These grants are available only to non-profit organisations and support basic research and early development of models and methods. CAAT has also served as a coordinator and funds administrator for special industry funding of specific 3R's topics.

Animal protection organisations

Historically the money available from the traditional animal protection organisations for 3R's research was quite small relative to government and industry spending. On the positive side, however, these funds were generally earmarked directly for 3Rs purposes and presumably had significant effect on the growth of alternative methods. Individual grants were often in the $5-10,000 range per year. It is encouraging that within the last two years more money is being made available to support general alternatives programs (e.g. 5 animal protection groups are major supporters of the U.S. Institute for In Vitro Sciences) as well as validation projects for specific *in vitro* assays. An excellent description of the various funding programs available for alternative research has been published in booklet form (Tarzi and Orlans, 1995).

Developers of commercial products for alternatives

Substantial contribution to the general field of alternatives has been provided by the commercial developers of tissue models, test kits, etc. which are used to conduct alternatives research and testing. Although most of these funds have not been available for competitive application, they have significantly increased the pace of alternatives progress. These funds have supported not only the development of their model, but also various groups conducting research in the general area of interest to that company. Although it is difficult to estimate the number of dollars spent on such activities, it is reasonable to assume that over $10M has been provided over the last ten years by companies like In Vitro International, Advanced Tissue Sciences, Organogenesis and MatTek.

Conclusions

The amount of money available in the U.S. for basic research that can lead to the development of alternative methods is satisfactory. Some money is available for specific test development through the SBIR program of the U.S. Government. However, it is only recently that the important activities of prevalidation and validation are being recognised as items that need support. It is hoped that government, industry and animal welfare can begin to supply funds. Nonethe-less, since there is no central overview of alternative funding activitie in the U.S., it is possible the promising methods will be "lost", failing to enter into the requisite validation studies.

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Institutions funding research according to the 3Rs in Germany

Summary

The largest programme in Germany with the aim of supporting research projects according to the 3R concept is being conducted within the Federal Governments biotechnology funding programme in the funding priority „Methods replacing animal experiments" of the Federal Ministry of Education and Research (BMBF). At present, DM 9.5 million is made available annually by the BMBF for this support measure.
Since 1990 ZEBET has been funding projects according to the 3R concept. In the last years, DM 650,000 have been expended by ZEBET annually for this purpose.
In a special funding programme, the federal state of Baden-Württemberg makes available DM 500,000 per year to support the development of alternative methods.
Another funding programme in this field in Germany is being conducted by the „Foundation for the Promotion of Research on Replacement and Complementary Methods to Reduce Animal Testing" (SET). For this purpose, „SET" provides about DM 500,000 per year, of which DM 400,000 is made available by the participating industrial associations.
In addition, the development of alternative methods is promoted in Germany by several research awards from foundations, associations and national authorities.

Keywords: 3R, research funding, Germany

Zusammenfassung: Forschungsförderung im Sinne des 3R-Konzeptes in Deutschland
Das grösste Programm zur Förderung von Forschungsvorhaben im Sinne des 3R-Konzeptes wird in Deutschland im Rahmen des Biotechnologie-Förderprogramms der Bundesregierung im Förderschwerpunkt „Ersatzmethoden zum Tierversuch" des Bundesministeriums für Bildung und Forschung (BMBF) durchgeführt. Zur Zeit werden jährlich 9,5 Mio. DM

vom BMBF für diesen Förderschwerpunkt zur Verfügung gestellt.
ZEBET fördert seit 1990 Forschungsvorhaben im Sinne des 3R-Konzeptes. In den letzten Jahren liegen die Ausgaben bei ca. 650.000.- DM pro Jahr.
Das Land Baden-Württemberg unterstützt in einem besonderen Förderschwerpunkt Forschungsvorhaben mit dieser Zielsetzung. Dafür werden pro Jahr 500.000.- DM zur Verfügung gestellt.

Ein weiteres Förderprogramm wird in Deutschland von der "Stiftung zur Förderung der Erforschung von Ersatz- und Ergänzungsmethoden zur Einschränkung von Tierversuchen" (SET) durchgeführt. Für diese Aufgabe werden pro Jahr etwa 500.000.- DM eingesetzt, von denen 400.000.- DM von den beteiligten Industrieverbänden zur Verfügung gestellt werden. Darüber hinaus wird die Entwicklung von Alternativmethoden in Deutschland durch mehrere Forschungspreise von Stiftungen, Verbänden und Behörden unterstützt.

Funding institutions

The largest research support programme in Germany for the development of alternative methods is being conducted within the framework of the Federal Government's biotechnology funding programme in the funding priority "Methods Replacing Animal Experiments" of the Federal Ministry of Education and Research (BMBF). The BMBF currently makes DM 9.5 million available annually for this support measure alone. Later this funding programme will be discussed in more detail.

ZEBET supports research projects on the scientific investigation of methods for replacing animal experiments since 1990. In the last years approx. DM 650,000 have been expended by ZEBET annually for this purpose.

Support is primarily given to the development and standardisation of new tests with the aim of creating the prerequisites for subsequent validation in national and international programmes. Many of the working groups supported by ZEBET have been awarded national and international research prizes for developing alternative methods.

In a special funding priority of its Ministry of Science, Research and the Arts, the Federal State of Baden-Württemberg is also supporting research projects with this goal. DM 500,000 is made available annually for this purpose. Funding is only given to universities in Baden-Württemberg with the goal of supporting the development of alternatives to frequently performed animal experiments in which animals are exposed to high levels of stress or where a large number of animals are used, including the educational sector.

Another funding programme in this field in Germany is being conducted by the "Foundation for the Promotion of Research on Replacement and Complementary Methods to Reduce Animal Testing" known as "SET" for short. SET was founded in 1986 on the initiative of the Federal Ministry of Food, Agriculture and Forestry. SET has a special character in that animal protection organisations and industrial associations work together on an equal footing.

In addition to research projects on developing alternatives to animal testing, SET also funds publications and training courses in this field in order to encourage a widespread application of alternative methods. Within the framework of research funding, priority is given to projects in areas where experiments in which animals are exposed to high levels of stress are still performed. A total of about DM 500,000 per year is provided for these tasks by SET, of which DM 400,000 is made available by the participating industrial associations.

SET's research funding represents a valuable complement to public research promotion in Germany. Its advantage is its flexibility and the possibility of funding small promising projects which do not, or not yet, fit into other research programmes. Thus projects can be initiated, which can then be continued in other programmes for the development of alternative methods.

Mention should also be made here of the research awards for promoting research on the replacement or reduction of animal testing. In 1992 National and European research awards were listed in a study by the Animal Protection Academy of the German Association for Animal Protection in cooperation with ZEBET. This study lists 6 research awards for Germany specifically concerned with this aim. They are awarded by foundations and associations, such as the Felix Wankel Foundation, or by national authorities. The corresponding research prize announced by the Federal Ministry of Health was awarded for 1998 at the start of this symposium.

BMBF funding

The BMBF's first mentioned funding priority "Methods Replacing Animal Experiments" shall now be discussed in somewhat more detail:

The first research projects with the central aim of replacing animal experiments were supported in 1980 by the BMBF's biotechnology funding programme. However, up to 1984 there was no special research funding programme on this topic. In 1984 a separate funding priority with this aim was set up within the biotechnology funding programme. The funding priority was then continued in May 1989 and June 1998 with more strongly application-oriented announcements.

The aim of the funding priority is to make as effective a contribution as possible to replacing animal experiments by promoting the development and validation of alternative methods. The funding programmes have been realised focusing on reduction and replacement. The goals of the 3R concept were then specifically included in the currently applicable announcement of June 1998.

Priority is given to the funding of application-oriented projects with the aim of developing or validating alternatives to animal experiments involving high levels of stress or where a large number of animals are used. In recent years, particular emphasis has been given to the development and validation of alternatives to animal experiments which are required by regulations.

Projects supported in this funding priority should have good prospects of wide-

spread, in particular industrial, application with a high potential for replacing animals testing according to the 3R concept. The projects should therefore be conducted with user participation, that is to say if possible together with industrial companies. This should ensure application-oriented handling and improve the prospects of widespread application of the methods derived.

Since the funding priority was established, projects have been supported on almost all topics of relevance. Three projects, all related to animal experiments which are required by regulations will be mentioned here as examples.

Thus for example, a validation study on replacing the Draize test on the rabbit eye co-ordinated by ZEBET / Prof. Spielmann was implemented within the funding priority. It was successful in ensuring that the testing of extremely irritating substances in Germany no longer needs to be carried out on the rabbit eye.

In another validation study coordinated by the Federal Institute for Health Protection of Consumers and Veterinary Medicine, the so–called ATC method for determining the acute oral toxicity of chemicals was successfully validated. The ATC method reduces the number of experimental animals by about 70% on average in comparison to the LD_{50} test. The new testing method was officially recognised by the OECD and the EU in 1996 and can thus be employed world-wide.

As part of the BMBF funding priority, numerous research projects have been conducted in the past few years on replacing animal experiments in the quality and efficacy control of immunobiologicals. These projects were all conducted in coordination with and generally with the participation of the German control authority for immunobiologicals, the Paul Ehrlich Institute in Langen.

The results of the project "Research on the Relevance of the Requirement V.2.1.5 of the German Pharmacopoeia on Testing for Abnormal Toxicity of Vaccines" led to the regulation on testing for abnormal toxicity being deleted from most vaccine monographs. This saves about 20,000 guinea pigs and mice per year.

Altogether 221 research projects were funded from 1980 to 1998 within the framework of the BMBF funding priority „Methods Replacing Animals Experiments" and were supported by a total of DM 135 million. Currently DM 9.5 million is made available each year by the BMBF for this funding priority.

Number of animals reduced by more than 40% since 1989

From 1989 to 1996 the total number of animals used in experiments in Germany dropped by more than 40%. However, with respect to the 3R concept, more has been achieved because the statistics on the number of animals used does not reflect „refinement", since the latter is not necessarily associated with a reduction in the number of experimental animals.

The research funding by the institutions mentioned above has made a considerable contribution to the reduction in the number of experimental animals in Germany. It should, however, be mentioned here that this drop is also principally due to the independent efforts of the industrial companies involved and other research institutions in Germany at developing and applying alternative methods wherever feasible in order to reduce the number of animal experiments as far as possible.

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Research Funded by ZEBET in the Past 10 Years

Johannes Doehmer, N. Krebsfänger, W. Schober, A. Luch, and J. T. M. Buters
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The establishment of the V79 cell battery and its application in toxicology and pharmacology

Abstract

New approaches offered by biotechnology is surely setting the stage for more direct involvement of scientific issues underlying the testing of chemicals in the near future. The potential of cellular and molecular models as replacements for long-standing animal models in testing protocols for chemicals has been recognised recently by regulatory agencies, e.g. the FDA and EPA in the United States. The application of biotechnology and genetic engineering is particularly indicated in the area of biotransformation of chemicals, where enzymes play a crucial role in the toxication of chemicals or the pharmacological efficacy of drugs. The power of gene technology is given by the cloning of genes encoding those enzymes and heterologous expression in a cellular environment much better suited for experimental use than the native genetic environment. In this way, species barriers can be easily crossed and compared. The key enzymes in biotransformation are the cytochrome P450 responsible for the initial metabolic activation of most chemicals. For risk assessment and safety reasons it is important to identify the enzymes involved and metabolites formed and the resulting toxic effects. Several cytochrome P450 from man, rat, mouse and fish have been cloned and inserted into vectors for expression in V79 Chinese hamster cells. The V79 cell line was initially chosen for two fundamental reasons. First, the V79 cell line is well

*established in toxicology for the testing of various endpoints. V79 cells have biological features not given with any other cell line, including human cell lines. Their karyotype and morphology is stable allowing for gentoxicity studies with extremely low background aberrations. Second, V79 cells do not express endogenous cytochrome P450. Therefore, genetically engineered V79 cells are defined for the cDNA encoded cytochrome P450 making them an analytical tool in metabolism related studies on chemicals and drugs. The establishment of the V79 cell lines started with the expression of rat cytochrome P450 in 1986, and was continued in 1990 for expression of human cytochrome P450 and later those of mouse and fish. The latest addition are V79 cell lines expressing the cytochrome P450 2D6 highly polymorphic in humans. All five known enzymatically active isoforms *1, *2, *9, *10 and *17 are expressed. As usual, the cDNAs are stable integrated into the V79 cell genome for stable expression demonstrated by Western analysis,* in situ *immunofluorescence, and enzyme activity as bufuralol-1-hydroxylation. Very recently, a CO-difference spectrum was obtained allowing for exact quantitation of cytochrome P450 in V79 cells. Although cytochrome P450 2D6 makes only 1% of all cytochrome P450 in the human liver, more than 30% of all drugs are being metabolised by this form. The most important application of those newly established cell lines will be in the pharmacokinetic testing of newly developed drugs in dependence of the polymorphic form, allowing for an individual dosing regimen of cytochrome P450 2D6 metabolised drugs. This has been recently exemplified and demonstrated for tamoxifen.*

Keywords: 3R, replace, V79 cell battery, gene technology, cytochrome P450, pharmacokinetic testing

Acknowledgement
The initial construction of the human cytochrome P450 expressing cell lines was funded by ZEBET for 3 years: 1990-1992. Current support by the BMBF is gratefully acknowledged. All V79 cell lines are being currently pre-validated by Dr. S. Coecke and collaborators (ECVAM) within an EU funded contract. Last but not least the valuable co-operation in the construction of the cytochrome P450 2D6 cell lines and the testing of tamoxifen by Drs. U. Zanger and M. Eichelbaum and collaborators (Dr. Margarethe-Fischer-Bosch-Institut, Stuttgart, Germany) should not go unnoticed.

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Kaomei Guan, Michael M. Schmidt, Qing Ding, Hong Chang, and Anna M. Wobus
In Vitro Differentiation Group, IPK, D-Gatersleben:
Embryonic stem cells *in vitro* - prospects for cell and developmental biology, embryotoxicology and cell therapy
This contribution is presented as an article in ALTEX 3/99 (135-141).

Rüdiger Schade
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Egg yolk antibodies (IgY) as an alternative to polyclonal mammalian antibodies. The history of the achievements of a joint project on IgY-technology in Germany

Abstract
This article is about the aims and achievements of a joint project on IgY-technology from 1992 to 1997. A lot has already been written about this technology's potential, so this time we shall be glancing back. How has the situation as regards avian antibodies changed over the last few years?
The first steps towards a joint project on an alternative way of gaining antibodies were taken in 1990, shortly after the fall of the Berlin wall. Contacts were established between representatives of three institutes: H. Spielmann, ZEBET (Centre for Documentation and Evaluation of Alternatives to Animal Testing), BgVV (Federal Institute for Health Protection of Consumers and Veterinary Medicine); C. Staak, BgVV; R. Schade, Charité, Humboldt University).
In former East Germany the theme of animal protection had been treated more pragmatically than in the west, where debates had been very emotional, so alternatives to experiments on animals had drawn little attention. None the less the extraction of antibodies from the yolk of hens' eggs was a well developed procedure at the Institute for Pharmacology and Toxicology at the Charité in former East Berlin, so there was a good basis for a joint project with institutions in the western part of the city, where this technology had been used for years. Through several lectures and publications (Schade et al., 1991) the ground for a joint project was cleared, and the nature of this phase of preparation was decisively influenced by ZEBET. A number of groups were then won over as partners: A group in Leipzig (H. Fiebig, I. Behn) had been researching for years into the phylogenesis of the immune system of vertebrates, so was familiar with non-mammalian antibodies. A group from the BgVV (C. Staak) had years of experience in the field of avian antibodies (IgY). A group from the Faculty for Veterinary Medicine at Berlin's Free University (A. Hlinak) was

likewise working with yolk antibodies and had contacts to the former Agricultural Teaching and Research Centre at Berlin's Humboldt University, where research was being done into SPF henkeeping (directed by H. Kobilke), and where facilities were then made available for essential project tasks.
With support from ZEBET, an application for a project was formulated and sent to the former German Ministry for Research, Education and Technology (BMFT). The application was approved, so the project began in 1992.
At this time, the situation as regards IgY-antibodies was as follows:
Although the existence of egg-yolk antibodies had been known since the work of Klemperer in 1893, the extraction of such antibodies for diagnostic purposes was generally ignored, and only single cases of the successful immunisation of hens were being reported, though this was a rising trend (Schade et al., 1991) due partly to more public debate about cruelty to animals. Knowledge about this methodology was meagre, so there was a lot of unjustified prejudice and discouraging misinformation.
The method could not be used easily, since suppliers offered little back-up in the form of secondary reagents, and none in the form of practical IgY-extraction kits.
In view of these facts, the project focused on new and further developments in the field of methodology. The results were data on the immunisation of hens, on laying performance after immunisation and on extraction procedures, as well as comparisons of IgY-preparation procedures, and data on the effectiveness of IgY-antibodies (see ALTEX 13, *(1996) Suppl.).*
These results were so persuasive that an application for support for a second phase of research was sent to the same ministry and likewise approved. Once more, essential support was given by ZEBET.
The first phase of the joint project (1992-1994) was marked by intense group efforts to inform the public of achievements in the field. The Berlin group, for instance, was interviewed on television about alternative antibodies, and the interview (Forscher, Fakten, Visionen - Researchers, Facts and Visions) was shown twice, the first time being on Bavarian Television in February 1993, and the second time on Satellite 3 later the same year. Due to these efforts, the interest of researchers in similar fields grew.
The second phase of the project (1994-1997) was marked by group efforts towards showing the effectiveness of IgY. They also won the support and co-operation of a group represented by U. Lösch and M. Erhard at the Ludwig-Maximilian University in Munich. In particular, hens were shown to be especially suitable as a source of antibodies against "problematic antigens" like lipopolysaccharides or antigens highly preserved during phylogenesis. A crucial aim was to show a difference between the specificities of antibodies from hens and rabbits, in spite of use of the same antigen (i.g. Gerl et al., 1996). Arguments in favour of IgY-technology widened to embrace not only the prevention of cruelty to animals but also the proven efficacy of the method (Schade et al., 1997).
This emphasis on the efficacy of the approach seems to have contributed crucially towards its being accepted.
Highlights of the project were two international meetings in 1996: One of these was a symposium about IgY-technology, in which the results of the project itself and of research with Behring AG (Doth et al., 1996), as also with Bayer AG (Gerl et al., 1996), were presented. The other was an ECVAM-workshop, leading to recommendations which are now internationally cited (Schade et al., 1996).
The project ended in 1997, and the benefits are shown in table 1. The willingness and opportunity to use IgY-antibodies in diagnosis, both routine and experimental, in 1999 may be shown as follows:
IgY-technology is now recognised internationally as an alternative to experiments on animals (Guidelines for the Protection of Cruelty to Animals 3.04, Swiss Federal Office for Veterinary Medicine, 3097 Liebefeld-Bern, 8th March, 1999, ho-800.116-3.04). As late as 1992, this theme was still controversial. Recommendations in the use of IgY-technology have been formulated by international experts (Schade et al., 1996).
There has been a notable growth of interest in IgY-antibodies as well as in IgY-technology, and by now there are commercial suppliers of the former (e.g. BIOGENES, GERBU).
There is now much more back-up for the use of IgY-technology, and there are several firms supplying marked secondary antibodies in various forms (e.g. SIGMA, DIANOVA and BioGenes). Likewise there are now extraction-kits based on various procedures like precipitation-techniques and affinity chromatography (SERVA, CLONTECH, BIOTEZ Berlin-Buch). Special cages for keeping hens in conditions suitable to their nature are available from various suppliers (EBECO, EHRET).
All this shows clearly that attitudes towards the use of avian antibodies in routines and research have basically changed, thanks partly to the efforts of the project groups. In 1997 their contribution to the development of IgY-technology was publicly acknowledged: they were awarded the European FISEA-Prize for successful research in the field of alternatives to experiments on animals.

Keywords: 3R, refine, IgY, chicken antibody, IgY-technology

Kurzfassung: Eidotter Antikörper (IgY) als eine Alternative zu polyklonalen Säuger-Antikörpern.
Geschichte und Bilanz eines IgY-Technologie Verbundprojektes
Im Jahre 1990 trafen sich Vertreter von vier Laboratorien, zwei davon aus ehemals Westdeutschland (Prof. Staak vom BgVV Berlin und Dr. Hlinak von der Freien Universität Berlin) und zwei aus ehemals Ostdeutschland (Dr. Behn von der Universität Leipzig und PD Dr. Schade von der Charité, Humboldt-Universität zu Berlin), um eine Kooperation auf dem Gebiet der alternativen Antikörper (Ak, in diesem Fall Ak aus den Eidottern immunisierter Hühner) zu erreichen. Mit wesentlicher Unterstützung durch ZEBET wurde eine Zusammenarbeit dieser vier Gruppen im Rahmen eines vom damaligen Bundesministerium für Forschung und Technologie (BMFT) geförderten Verbundpro-

*jektes, das 1992 begann, verabredet. Die IgY-Technologie ist eine reale Alternative zur Gewinnung von Säuger-Ak im Sinne der 3R (*replace, reduce, refine*), da die IgY-Ak unblutig aus dem Dotter präpariert werden können. Zu Beginn des Projektes gab es nur eine geringe Akzeptanz der IgY-Ak, zurückzuführen auf mangelnde Information, Vorbehalte sowie auf eine fehlende oder wenig entwickelte "Peripherie" (markierte Sekundär-Ak, IgY-Präparations-Kits). Das Projekt konzentrierte sich daher vornehmlich auf methodische Entwicklungen.*
Die in diesem Projekt gewonnenen Ergebnisse waren so überzeugend, daß eine zweite Förderphase beantragt und auch genehmigt wurde, wiederum mit substantieller Unterstützung seitens ZEBET. An diesem Projekt waren fünf Arbeitsgruppen beteiligt (zusätzlich Prof. Lösch, Dr. Erhard von der Ludwig-Maximilians-Universität, München). Da das Interesse der Wissenschaftler an der Praktizierung der IgY-Technologie deutlich gestiegen war, war das Projekt diesmal eher anwendungsorientiert.
Im Rahmen des Projektes wurden 44 Vorträge gehalten und 33 Poster ausgestellt, es entstanden 58 Publikationen in verschiedensten Zeitschriften und Kongressbänden. Ende 1999, Anfang 2000 wird ein Laboratory Manual *über die IgY-Technologie vom Springer-Verlag herausgegeben. 1996 fanden in Berlin ein internationales Symposium sowie ein ECVAM-Workshop zum gleichen Thema statt. Sechs Nachwuchswissenschaftler promovierten mit Themen zur IgY-Problematik.*
Im Vergleich zum Beginn des Projektes hat sich die Situation bezüglich der IgY-Ak erheblich gewandelt:

- *Die IgY-Technologie ist als alternative Methode anerkannt, einige Jahre zuvor wurde dies noch kontrovers diskutiert.*
- *Es besteht ein ausreichendes Angebot an markierten Sekundär-Ak und IgY-Extraktions Kits verschiedener Anbieter.*
- *Es gibt verschiedene Biotechnologie-Unternehmen, die die Gewinnung von IgY-Ak als Service anbieten.*

Das IgY-Projekt hat an dieser Entwicklung wesentlichen Anteil. 1997 sind die Aktivitäten der am Projekt Beteiligten mit dem europäischen FISEA-Preis für Arbeiten zu Alternativen zu Tierexperimenten ausgezeichnet worden.

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New applications of the human whole blood pyrogen assay (pyrocheck)

This contribution is presented as a short communication in ALTEX 3/99 (146-149).

Augustinus Bader, Angelika Langsch, Ute Weingartz, Karin Burgwitz, and Axel Haverich
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Bioartificial thin section liver analogues maintain cytochrome P450 induction properties and can be cryopreserved

Abstract

Knowledge of drug biotransformation pathways at early stages of drug development could considerably speed up drug development and increase the safety of drug development in the pharmaceutical industry. Maintenance of cytochrome P450 pathways and cryopreservation potential represent crucial aspects for a generic use as a model in preclinical drug development.

Materials and methods: Thin section analogues of the liver were artificially reconstructed intending to mimic the physiologic microenvironment of the liver in a small scale and accessible form. Drugs specific for phase I and II metabolism were added to investigate the maintenance of biotransformation capacities over two weeks in culture. Porcine and human primary liver cells were studied. All experiments were repeated at least three times. Metabolites were analysed by high performance liquid chromatography or fluorescence. Cultures were cryopreserved and evaluated for P450 activities upon thawing.

Results: Bioreactor cultures are advantageous over static Petri dish systems since they can be better controlled and

thus standardised with respect to nutrient and oxygen supply. A mini-bioreactor device for the organotypical culture of reconstituted liver tissue has been developed. Phase I (CYPs 1A, 2B, 3A) and II metabolic acitivities (UGT, ST, GT) were maintained at high and stable rates over the study period (2 weeks). Strong chemical induction using typical inducers was detected. Cultures maintained benzodiazepine metabolism comparable to not cryopreserved controls over the full study period. Conclusions: In the present study the development and biochemical characterisation of an organotypical thin section cell culture model of the liver is described. The model is useful for drug metabolism studies but maintains also typical hepatospecific properties. Crucial parameters required for a generic use as a drug metabolism model, including cryopreservation capacity and cytochrome P450 induction, are maintained.

Keywords: 3R, replace, drug metabolism, liver, culture

Kurzfassung: Bioartifizielle Leber-Dünnschnitt-Analoge behalten die Cytochrome P450 Induktion bei und können kältekonserviert werden

Die Beibehaltung der Induktionsfähigkeit biotransformatorischer Enzyme und die Kryokonservierbarkeit stellen Schlüßelanforderungen an ein Kultursystem für eine generische Anwendung des Modells in der präklinischen Entwicklung von Arzneimitteln dar.
Arbeitsprogramm: Leberzellen wurden unter Rekonstruktion des Disseschen Spaltraums und mit nicht-parenchymalen Zellen kokultiviert. Methodiken für die Untersuchung der Induktion des Phase I und II-Stoffwechsels wurden mittels Standardinduktoren an porkinen und menschlichen Leberzellen aufgebaut und in initalen Untersuchungen evaluiert. Metaboliten wurden mittels HPLC und fluorometrischer Verfahren identifiziert. Ein Einfrierprotokoll wurde entwickelt.
Aktueller Stand der Arbeiten: Die analytischen Verfahren wurden in Abstimmung mit mehreren großen pharmazeutischen Unternehmen entwickelt und in diesen Betrieben etabliert. In den Kultursystemen blieben in den porkinen Sandwichkokulturen die metabolischen Aktivitäten der Phase I (CYPs 1A, 2B, 3A) und II (UGT, ST, GT) auf hohem Niveau erhalten. Erste funktionsfähige Protokolle für die Kryokonservierung von Sandwichkokultursystemen wurden entwickelt und getestet.
Schlußfolgerungen: In dieser Studie wurde gezeigt, daß organotypische Leberzellkulturen in einem als Sandwichkokulturen auch in längerfristiger Kultur metabolische Aktivitäten erfolgreich hinsichtlich Phase I und II sowie Induktionsfähigkeit untersucht werden können. Erste Kryoversuche waren erfolgreich. Bioreaktor-Kulturen besitzen im Vergleich mit Petrischalen praktische Vorteile hinsichtlich besserer Kontrollier- und Standardisierbarkeit der Verfügbarkeit von Sauerstoff- und Nährstoffen. Das optimierte Modell beruht auf einer Rekonstruktion der 3-D Mikroumgebung der Leberzellplatten unter Berücksichtigung der physiologischen Geometrie extrazellulärer Matrix und einer Kokultivierung nicht-parenchymaler Zellen (Leberzellplattenmodell). Das Modell liefert zuverlässig für den Menschen prädiktive Ergebnisse, wie sie bisher in den klassischen Tierversuchen auf Grund speziesabhängiger Enzymausstattungen im P450 System nicht immer erzielt werden konnten.

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Manfred Kietzmann
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The isolated perfused bovine udder as a model of transdermal penetration and absorption of drugs

Abstract
Because studies with non-perfused skin are of limited predictive value for pharmacokinetic studies of the rate of transdermal penetration, permeation and absorption, the isolated perfused bovine udder was introduced as an in vitro *model of transdermal penetration and absorption (Kietzmann et al., 1993). Udders from slaughtered healthy cows are used for the experiments. Bovine udders are obtained immediately after the slaughter of healthy cows. The udders are perfused with oxygenized tyrode solution (38.5°C). The period of perfusion starts within 30 minutes after slaughtering. The organ is supplied by the cannulated* arteria pudendalis externa *with a venous drainage via the vena epigastrica cranialis superficialis. The viability of the perfused udder skin was demonstrated by a nearly unchanged glucose consumption, an initially decreasing and thereafter unchanged lactate production and an unchanged lactate dehydrogenase activity.*
First studies were performed to demonstrate the transdermal absorption of various test compounds like dexamethasone, benzyol peroxide and etofenamate. In these studies, drug concentrations were measured in perfusate fractions only. Using betamethasone-17,21-dipropionate containing formulations as test compounds, the influence of galenic formulations on the penetration and absorption of active compounds was demonstrated. Compared with an alcoholic solution, the absorption rate was significantly enhanced after administration of betamethasone-17,21-dipropionate as an ointment. The

importance of the barrier function of the horny layer was confirmed also. After disruption of the horny layer by repeated administration of acetone, a significant increase of the glucocorticoid absorption was obvious after treatment with the ointment.
In addition to drugs which are used topical for the treatment of skin disorders, studies of transdermal penetration and absorption are important for topical administered drugs with systemic activity (transdermal therapeutic systems). Therefore, the transdermal bioavailability of isosorbide dinitrate (ISDN) was studied. Compared with ointment and spray, a significantly enhanced transdermal absorption of ISDN was measurable after topical administration of a patch containing the organic nitrate as a microemulsion. Using transdermal therapeutic systems containing estradiol-17β (patches), it was possible to describe the transdermal flux of estradiol-17β by measurement of the drug concentration in adhesive tape strips from the horny layer, in microtome sections of the viable skin and in perfusate samples.
The isolated perfused bovine udder seems to be a very suitable and predicative in vitro *system for comparative studies of transdermal penetration and absorption of topical administered drugs.*

Keywords: 3R, replace, transcutaneous penetration, bovine udder, dermal absorption

Kurzfassung: Das isoliert perfundierte Rindereuter als Modell zur Untersuchung der transdermalen Penetration und Resorption
Da Untersuchungen zur transdermalen Penetration, Permeation und Resorption von eingeschränktem prädiktiven Wert sind, wurde das isoliert perfundierte Rindereuter als in vitro *Modell für diese Fragestellungen eingeführt (Kietzmann et al., 1993). Euter geschlachteter Kühe werden für die Untersuchungen verwendet. Nachdem direkt nach der Schlachtung heparinisierte Tyrodelösung in das Euter infundiert wurde, wird das Organ in das Labor gebracht und dort mit angewärmter (38.5°C) und begaster Tyrodelösung über die* arteria pudendalis externa *perfundiert (Abfluß des Perfusats über die* vena epigastrica cranialis superficialis*). Die Perfusion beginnt etwa 30 Minuten nach der Schlachtung. Die Vitalität des Gewebes wird durch Messung des Glukoseverbrauchs, der Laktatproduktion und der Aktivität des Enzyms Laktatdehydrogenase im Perfusat belegt.*
In ersten Studien konnte mit dem in vitro *Modell die transdermale Resorption verschiedener Testsubstanzen (Dexamethason, Benzoylperoxid und Etofenamat) gezeigt werden. In diesen Untersuchungen wurde die Wirkstoffkonzentration lediglich im Perfusat gemessen. Unter Verwendung verschiedener Betamethason-17,21-dipropionat enthaltender Formulierungen wurde nachfolgend die Bedeutung der jeweiligen Darreichungsform (Salbe, Creme, Gel, Lösung) für die Penetration und Resorption von Wirkstoffen gezeigt. Im Vergleich zur alkoholischen Lösung des Glukokortikoids war die transdermale Resorption nach Verabreichung als Salbe, Creme oder Gel signifikant gesteigert. In dieser Studie wurde gleichzeitig die Bedeutung der Hornschicht als Barriere aufgezeigt. Die Störung der Barrierefunktion der Hornschicht durch wiederholte Applikation von Aceton führte zu einem signifikanten Anstieg der transdermalen Penetrations- und Resorptionsrate, wenn der Wirkstoff als Bestandteil von Salbe, Creme oder Gel verabreicht wurde.*
Zusätzlich zu Arzneimitteln, die topisch zur Behandlung von Hauterkrankungen eingesetzt werden, spielen sogenannte transdermale therapeutische Systeme eine zunehmend wichtige Rolle. Auch für diese Arzneimittel sind Untersuchungen zur transdermalen Resorption besonders wichtig. Beispielhaft wurde die transdermale Resorption von Isosorbiddinitrat (ISDN) untersucht. Im Vergleich zu ISDN-haltigen Salben und Sprays zeigte ein transdermales System, welches ISDN in einer Mikroemulsion enthält, eine signifikant höhere Penetrations- und Resorptionsrate. Unter Verwendung des isoliert perfundierten Rindereuters wurden ebenfalls verschiedene Estradiolpflaster verglichen. Dabei wurde die Hormonkonzentration zusätzlich zum Perfusat auch in Tesafilm-Abrissen und in Gefriermikrotomschnitten der Haut (Messung der Wirkstoffkonzentration in der Hornschicht beziehungsweise im Hautgewebe) ermittelt. Mit Hilfe dieser Daten konnte der transdermale Flux des Wirkstoffes sehr gut charakterisiert werden, so daß auch eine vergleichende Abschätzung der Bioverfügbarkeit möglich ist.
Zusammenfassend kann festgestellt werden, daß mit dem isoliert perfundierten Rindereuter ein in vitro *Modell für die Untersuchung der transdermalen Penetration und Resorption topisch verabreichter Arzneimittel zur Verfügung steht.*

Acknowledgement
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Support Provided by Information Services

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ZEBET, BgVV, Berlin:
The ZEBET database and information service

Summary
The ZEBET's Information Service provides its information primarily to the scientific and animal committees which are regulating the use of experimental animals according to EEC Directive 86 609 at the State and District level of the Federal Republic of Germany. ZEBET's Information Service will also answer information inquires from other institutions, the media, individual scientists and the general public. So fare, ZEBET's Information Service is not available as an on-line service or through the Internet, but has to be addressed to ZEBET by fax or mail. The ZEBET's Information Service not only consults the ZEBET database, but also the majority of on-line databanks, e.g. MEDLINE, EMBASE or TOXLINE. ZEBET's Information Service has responded to more than 1,800 inquiries. Of the 450 inquiries in 1998 approximately 50 % were submitted by industry, universities and scientific institutions.
The ZEBET Database was set up to document alternatives to the use of experimental animals from the international literature. The main concept of the ZEBET database is to evaluate and document alternative methods according to the „3R" concept of Russel and Burch (1959). Each document of a method contains a short description of a method and bibliographic references. In addition, an evaluation by the ZEBET staff indicates whether the method results in replacement, reduction or refinement of animal use. The documentation covers 300 methods, and more than 6,000 bibliographic references. Currently the ZEBET database is an in-house database. ZEBET is going to offer the database to the public through DIMDI, the German Institute for Medical Documentation and Information.

Keywords: 3R, database, information service, ZEBET, animal protection law

Zusammenfassung: ZEBET Datenbank und Informationsdienst
Im Rahmen des Vollzuges des Tierschutzgesetzes in Deutschland fertigt ZEBET auf Anfragen von Länderbehörden zu Anträgen auf Genehmigung oder Anzeigen von Tierversuchsvorhaben Gutachten an. Darüber hinaus beantwortet ZEBET Anfragen von Wissenschaftlern, Tierschutzbeauftragten und anderen Interessenten zur Anwendung von Ersatz- und Ergänzungsmethoden zu Tierversuchen. Die Anfragen werden in schriftlicher Form an ZEBET gerichtet. ZEBET nutzt für den Informationsdienst die eigene ZEBET-Datenbank, aber auch Recherchen in nationalen und internationalen biomedizinischen Literaturdatenbanken wie z.B. MEDLINE, EMBASE oder TOXLINE. Von 1990 bis 1998 wurden von ZEBET insgesamt 1800 Anfragen beantwortet. 1998 kam die Hälfte der insgesamt 448 Anfragen aus der Industrie, Universitäten und Forschungszentren.
ZEBET hat den Auftrag Ersatz- und Ergänzungsmethoden zum Tierversuch in einer Datenbank zu dokumentieren. Das entscheidende Kriterium zur Aufnahme einer Methode in die ZEBET-Datenbank ist die Bewertung, ob durch die Anwendung der Methode das Leiden der Tiere vermindert (Refinement) und die Anzahl der Versuchstiere reduziert (Reduction) wird oder Tierversuche ersetzt (Replacement) werden. Die Kriterien Replacement, Reduction und Refinement werden in Anlehnung an das „3R-Konzept" von Russel und Burch (1959) vergeben. Für jede Methode wird von Mitarbeitern der ZEBET ein Dokument angefertigt, das eine kurze Beschreibung und Bewertung der Methode als Alternativmethode und ein Verzeichnis der relevanten Literatur enthält. In der ZEBET-Datenbank liegen Unterlagen zu ca. 300 Ersatz- und Ergänzungsmethoden zu Tierversuchen in der experimentellen Biomedizin vor. Dazu gehören mehr als 6000 Literaturstellen. Gegenwärtig ist die ZEBET-Datenbank eine nur intern nutzbare Datenbank. Sie wird zukünftig über das Deutsche Institut für Medizinische Dokumentation und Information (DIMDI) online zugänglich sein.

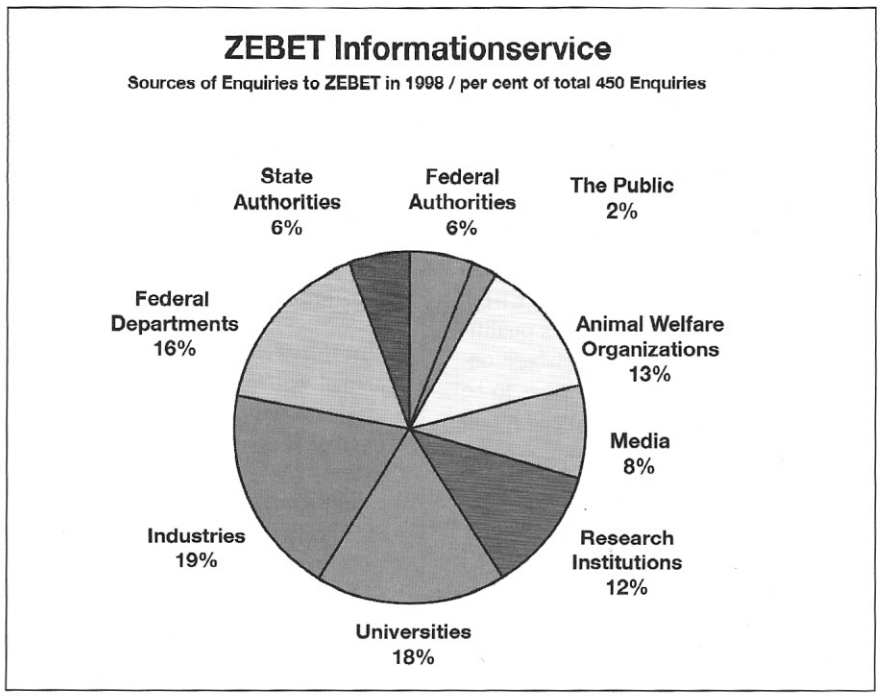
The ZEBET Database and Information Service

In the process of harmonizing the German Animal Protection Law (Tierschutzgesetz) within the European legislation (Council Directive 86/609/EEC), the Centre for Documentation and Evaluation of Alternative Methods to Animal Experiments (Zentralstelle zur Erfassung und Bewertung von Ersatz- und Ergänzungsmethoden zum Tierversuch, ZEBET) was established in 1989. ZEBET is part of the Federal Institute for Consumer Health Protection and Veterinary Medicine (Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, BgVV). ZEBET is responsible for the documentation of alternative methods and also for the validation and acceptance of alternatives at national and international levels, e.g. by the EU and OECD.

Over the past 10 years ZEBET has served as an national and international information centre for alternatives to animal experiments. The celebration of ZEBET´s 10 Year Anniversary gives an opportunity to outline the experiences gained during this period at ZEBET. ZEBET with it's specific

information service and database works to enforce animal protection concerning the following requirements of the German Animal Protection Law:

- Replace, reduce or refine animal experiments.
- Prove that the scientific result can only be achieved by performing animal experiments.
- Consult all sources of information in order to prevent unnecessary animal experiments.



The ZEBET Information Service

ZEBET's Information Service provides information on alternative methods primarily to the local state and federal authorities in Germany, and in addition to local animal protection officers, scientists at universities, industry and research institutions, the general public, and journalists. Each of these groups of customers has separate and distinct objectives involving specific and unique situations. On the one hand ZEBET's Information Service gives advice to scientists and on the other hand ZEBET also serves as the official expert in Germany in controversial court decisions.

ZEBET's Information Service has responded to more than 1,800 inquiries from 1990 to 1998. It is imperative that ZEBET gives support to scientists both in industry and universities. Among the 450 inquires in 1998 approximately 50 % were submitted by the industry, universities and research institutions, as is shown in figure 1. The number of requests for information to establish an *in vitro* method continues to increase in every sector. The reasons for this increase are the international validation studies in which ZEBET has established itself as one of the internationally leading institutions as well as the close connection ZEBET is maintaining to the management teams of ongoing validation studies. ZEBET is committed to provide the most accurate information possible.

Each request for information has to be submitted in writing. ZEBET's staff responds individually to each inquiry and is consulting the ZEBET Database and other sources, e.g. reports, protocols and literature accessible through ZEBET´s connection to validation projects and to membership in expert committees, e.g. the German Standardisation Institute (DIN e.V., Berlin), searching national and international biomedical literature and factual databases via the German Institute for Medical Documentation and Information (DIMDI), information retrieved from the internet and confidential information e.g. draft test guidelines from the EU and OECD.

ZEBET's staff is trained in the biomedical sciences, as well as in searching strategies in open access databases. Information on a specific problem in the field of alternative methods is evaluated by scientists of the ZEBET staff. ZEBET also co-operates with other Federal Institutions in Germany, especially with the German Federal Institute for Drugs and Medical Devices. The document, which is sent to the inquirer includes print-outs from the ZEBET Database, and references from external databases.

The ZEBET Database

In addition to the individual Information Service, ZEBET is documenting alternative methods in the database in English language. ZEBET will in very near future offer this database on-line through DIMDI, the German Institute for Medical Documentation and Information. Like the BgVV, DIMDI is a federal institute reporting to the Federal Ministry of Health (BMG). DIMDI is offering a broad collection of databases covering the entire spectrum of the life sciences and the social sciences to its subscribers. DIMDI can be entered via all major communication networks. Additionally, anyone can use DIMDI´s service via the internet. Subscribers to DIMDI can search in databases with DIMDI´s powerful retrieval system *grips*, with *grips*-commands or with the user guidance *grips*-Menu or in internet/www with the graphic user interface *grips*-WebSearch.

The main concept of the ZEBET Database is to evaluate and document alternative methods according to the "3-Rs" concept of Russell and Burch (1959): Replacement - Reduction - Refinement.

To maintain and update the ZEBET Database it is essential to collect constantly original scientific publications, newsletters, monographs and conference proceedings, from database searches for example in MEDLINE or EMBASE, and also directly from scientists. Scientists at ZEBET evaluate if an new or modified procedures may serve as an alternative to animal test and should be documented in the ZEBET-Database.

The first level of our information inventory is compiled of current publications that are assembled every day. This information is first titled for the method, then keyword and prepared for further database processing before it can be filed in the database for the first time. Today over 200 documents of methods are stored in the ZEBET Database at this first level. More than 6,000 bibliographic references are included from approximately 800 journals and other sources. The ZEBET Database covers a broad spectrum of biomedical sciences,

including the following subjects: pharmacology, toxicology, pharmacy, bacteriology, virology, food hygiene, parasitology, immunology, neurology, cancer research, and animal production.

Currently the ZEBET Database is operated as an in-house database on a local PC-network within the BgVV. ZEBET will offer the database to the public through DIMDI. For this purpose the ZEBET Database complies with DIMDI's qualification criteria for offering a database on-line. Therefore, each document has to be reviewed and evaluated. In the end it will contain the following information:

- the title of an alternative method, keywords and selected literature
- a short description of the method including the most important details (aim and principle)
- an evaluation of the extent of development of the method for the purpose of reducing, replacing, or refining a specific experimental procedure in laboratory animals
- an assessment of the stage of development, validation or regulatory acceptance of the alternative method according to the classification suggested by Balls et al. 1990.

To summarise, the second level of the ZEBET Database is the assessment of information on a specific method. Since 1997 three scientists supported by a special grant from the Ministry of Health have prepared approximately 100 English documents for DIMDI. The documents are currently awaiting on-line distribution. All of these documents have passed the final assessment stage.

To get an idea on the funding that ZEBET-Database needs to maintain the highest level of accuracy, a rough estimate is given. Each document costs about 1,500 DM when taking into account a minimum of 4 work-days of preparation by Ph.D. qualified scientists. As one can imagine the financial support is required if ZEBET is to continue to provide information at the level expected.

An example of a document from the ZEBET-Database is shown below to give a brief outline of the scope of a ZEBET-Database document. Detailed information about the definition of each of the datafields including searching strategies will be available through DIMDI and the BgVV when the ZEBET Database is global accessible (see http://www.dimdi.de or http://wwwi.bgvv.de).

ZEBET-Database
Method No. 186
Last Revision: **1998 - 12 - 02**
Section Headings: **Toxicology**

Title: Acute Toxic Class Method for testing the acute oral toxicity of chemicals as a replacement of the classical LD50-test

Uncontrolled Terms:
animal welfare / animal experiments / animal testing alternatives / reduction / number of animals / rats / OECD Guideline / Commission of the European Communities / commission directive / chemicals / hazardous chemicals / hazardous substances / hazard assessment / hazard classification / classification / toxicity classes / toxicity, single dose / effect, systemic / toxicity, acute, oral / LD50 / LD50 test / median lethal dose / Acute Toxic Class Method / ATC Method / stepwise procedure

Evaluation : **Reduction; Refinement**
Status: **Acceptance**
Regulation: OECD Guideline for Testing of Chemicals 423, adopted 22.03.1996, Commission Directive 6/54/EC of 30 July 1996 ...
Abstract:
Background
In the assessment and evaluation of the toxic characteristics of a substance, determination of acute oral toxicity is usually an initial step. Data from an acute study serve as ...

Method
As an alternative to the LD50-test, the principle of the Acute Toxic Class Method (ATC Method) is a stepwise procedure for the testing of acute oral toxicity of chemicals. ...

Remarks:
The Up-and-Down Procedure and the Fixed Dose Procedure are other alternatives to the traditional LD50- test. ...

Selected References
(for example)
Diener W., Mischke U., Kayser D. and Schlede E. (1995). The biometrical evaluation of the OECD modified version of the acute toxic class method (oral). *Archives of Toxicology 69*, 729-734.

DIMDI is currently establishing the ZEBET Database as a compatible database for on-line service. We hope to be able to offer this system functioning properly as soon as possible. It is the very personal ambition of each team member of the database staff to complete the development of the on-line ZEBET Database in English. It will then be possible to search in the ZEBET-Database for specific details of a given method and also for information on the regulatory acceptance of alternative methods. The bibliographic references of the ZEBET Database can be used for additional searching in literature databases.

Conclusions

ZEBET´s experience has shown, that one of the prerequisites for the documentation of alternative methods is the critical evaluation of all information available on these methods. It is one of ZEBET's central tasks to provide evaluated information on alternatives to animal testing. ZEBET´s database differs from conventional databases by demanding a high standard of critical evaluation. Using this evaluated information ZEBET is able to offer suggestions in order to solve specific problems of the replacement, reduction or refinement of testing in animals.

A partnership with both national and international organisations such as ECVAM in Italy, the Johns Hopkins Centre for Alternatives to Animal Testing (CAAT) in Baltimore (USA), the Animal Welfare Information Centre in Washington DC (USA), and the Akademie für Tierschutz in Germany will help to expand our database potential today and it allows future co-operation to develop a thesaurus of alternative methods and to establish an standard for databases on alternatives.

Acknowledgement
ZEBET´s 10 Year Anniversary is a very good opportunity, to thank the German Federal Government for supporting ZEBET´s endeavour and funding scientists, who are exclusively working on the evaluation and documentation of alternative methods.

Our very special thanks for the members of the ZEBET-Commission, especially Dr. Brigitte Rusche, head of the commission, for supporting the ZEBET Database. Without this support we would not have achieved the progress that we have made to date.

We also want to thank the scientists involved in writing new documents for the ZEBET Database. We are particularly indebted to Dr. Rauch, Dr. Behnk-Koblau, Dr. Box, and Dr. Scherkel for their excellent work as freelance scientists at ZEBET.

References

Russell, W. M. S. and Burch R. L. (1959). *The principles of humane experimental technique.* London: Methuen.

Balls, M., Blaauboer, B., Brusic, D., Fraizier, J., Lamb, D., Pemberton, M., Reinhardt, C. A., Roberfroid, M., Rosenkranz, H., Schmid, B., Spielmann, H., Stammati, A. and Walum, E. (1990). Report and recommendations of the CAAT/ERGATT workshop on the validation of toxicity test procedures. *ATLA 18*, 313-337.

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Draft Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals used in Safety Evaluation

OECD SERIES ON TESTING AND ASSESSMENT
Number 19

The purpose of this Guidance Document is to apply the principles of the 3Rs to the use of animals in regulatory toxicity tests. This document specifically addresses Refinement.
In 1994, an *ad hoc* Working Group was formed. Prof. David Morton drafted a background document which laid the groundwork for this OECD Guidance Document. The objective of the Guidance Document is to provide useful guidance and criteria for determining when an animal is in a moribund condition, or expected to become moribund, or experiencing significant pain and distress, and should therefore be euthanized.
On 19th-20th November 1998, a Nominated Expert meeting was held in Zeist, The Netherlands, to critique and redraft a guidance document taking into account comments received from member countries. The new draft represents the consensus of the nominated experts.
This publication is available electronically, at no charge:
(http://www.oecd.org/ehs/test/flags.htm)

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LATEST NEWS

Experts are invited to submit comments to their National Co-ordinator **no later than 1st of December 1999.**

Annett Janusch-Roi and Michael Balls,
ECVAM, Institute for Health & Consumer Protection, Joint Research Centre, European Commission, I-Ispra (VA):
The ECVAM scientific information service (SIS)

Summary
SIS was established in 1996 as one of ECVAM's high priority activities. SIS is designed as a unique information tool on alternative methods to animal experiments, with particular emphasis on non-animal test development and validation in relation to particular types of potential toxic hazard. SIS is characterised by factual *and* evaluated *information. In addition to full method descriptions, more-detailed information is made available, to permit interrogations from a variety of different points of view. SIS is structured into the following main sectors: 1) alternative methods at any stage of development and validation, including test method protocols for their use (dbAlm); 2) full details of validation studies (dbVas); and 3) the ECVAM Workshop and Task Force Reports. Until now, SIS covers in total 21 topics in the area of* in vitro *toxicity testing, for which 17 Method Summaries, 155 Specific Techniques (including the whole* INVITTOX *protocol collection), 493 Test Results for 368 Test Compounds, 17 Evaluation Studies, and 1065 Bibliographic References are available. A test version of SIS with a first set of data is expected to be*

made publicly available through the Internet early in the year 2000. Data retrieval is ensured in a uniform and user-friendly way (user guidance), to address the requirements of different user profiles. The progress made in the collection of information has been as a result of collaborations with various institutions in Europe. Furthermore, a project on the development of a thesaurus on alternative methods has been initiated, as an outcome of the first ECVAM Database Task Force meeting, held in March 1999.

Keywords: 3R, database, factual information

Zusammenfassung: ECVAM's wissenschaftliches-Informationszentrum SIS
SIS, ein Informationssystem über Ersatz- und Ergänzungsmethoden zu Tierversuchen, wurde 1996 als einer der wichtigsten Bereiche von ECVAM engerichtet. SIS ist eine Faktendatenbank und enthält bewertete Informationen, die nach feststehenden Kriterien zusammengestellt werden. SIS ist derzeit vor allem auf in vitro *Methoden zur Toxizitätsprüfung ausgerichtet. Es umfaßt nicht nur Methodenbeschreibungen, sondern beinhaltet auch sehr detaillierte Daten, was breitgefächerte und vielfältige Recherchen erlaubt. SIS wurde, entsprechend der Herkunft seiner Daten aus der wissenschaftlichen Literatur oder von offiziellen Validierungsstudien, wie folgt unterteilt: 1) Alternativmethoden, in ersten Ansätzen oder solche, die schon einem Validierungsprozeß unterzogen wurden, einschließlich der Protokolle, die ein Nachvollziehen der entsprechenden Methode erlauben (dbAlm); 2) vollständige Informationen zu Validierungsstudien (dbVas); und 3) ECVAM's Workshop und Task Force Reports. SIS enthält zur Zeit Informationen für 21 Anwendungsgebiete toxikologischer Ersatzmethoden. Es stehen insgesammt 17 zusammenfassende Beschreibungen von Methoden zur Verfügung. Weiterhin umfaßt SIS 155 spezielle Techniken (einschließlich der* INVITTOX *Protokolle), 493 Tests, die mit 368 Chemikalien durchgeführt wurden, 17 unterschiedlichste Validierungsstudien, und zusätzlich wird der Zugriff auf 1065 Literaturangaben ermöglicht. SIS pflegt enge Kontakte mit Instituten und Wissenschaftlern in Europa, die wesentlich zu den guten Fortschritten bei der Datensammlung beigetragen haben. Die erste Version von SIS wird der Öffentlichkeit über das Internet zum nächstmöglichen Zeitpunkt zugänglich gemacht. Benutzerfreundlicher Zugriff zu den Daten soll auch den ungeübten Datenbankbenutzer erreichen. Weiterhin, startete kürzlich ein Projekt, das durch die ECVAM Database Task Force initiiert wurde, in dem die Durchführbarkeit der Erstellung eines Thesaurus über Alternativmethoden geprüft wird.*

Why SIS?

The origin of SIS, created in 1996, is to be found in a Communication of the European Commission to Council and European Parliament in October 1991, pointing to a requirement in Directive 86/609/EEC about alternative methods. One of the duties of ECVAM, as defined by the European Commission, is the establishment, maintenance and management of a database on alternative procedures. To best achieve this objective and to support ECVAM in fulfilling its mission, SIS has been established as one of ECVAM's high priorities activities.

Features

SIS is characterised by *factual* (not only bibliographic references) and *evaluated* information. It is an information system on alternative methodologies to animal experiments, with particular emphasis on non-animal test development and validation in relation to particular types of potential toxic hazard. SIS has been created by ECVAM with the main aim of establishing a unique information tool providing information on alternatives at any stage of development and validation, coming from a wide range information sources. SIS is designed to provide information necessary for scientists and/or decision makers to enable them to evaluate the usefulness of a specific methodology for their own requirements. In fact, in addition to full method descriptions, much more-detailed information is provided (test compounds and the description of tests carried out with the documented methods, including test results, information on test systems, description of intralaboratory and interlaboratory assessment studies, full details on formal validation studies, register of user laboratories, etc.). This also permits interrogation from a variety of points of view.

According to the origin of information, from the scientific literature and related information sources, or from official formal validation studies, carried out, for example, under the auspices of ECVAM, SIS is structured into the following main sectors:

- alternative methodologies (dbAlm)
- validation studies (dbVas)
- ECVAM Workshop and Task Force Reports

dbAlm

This database includes information on alternative methodologies at any stage of development and validation, taken from the scientific literature, contacts with scientists, congress proceedings, ECVAM-funded studies and technical reports, and other related information sources. With the aim of providing detailed information on alternatives and to satisfy the requirements of different user profiles, the following main data sectors are included:

Method descriptions.

The methods are described at various levels, such as summary records and/or protocols, with information on the topic area, objectives and applications, experimental details (such as test system used, endpoint and endpoint detection), basic procedure, type of tested materials, data analysis procedures, state of development, validation or regulatory acceptance according to ECVAM's principles for test development and validation, analysed bibliography and reference to a brief description of the related *in vivo* test.

Test systems.

Descriptions of tests and their results, carried out with documented methods by various laboratories.

User laboratories.

N.B. The collection of *INVITTOX* protocols (Ungar, 1993), which provides step-by-step descriptions of *in vitro* methodologies, has been transferred from FRAME (UK) to ECVAM, and has become an integral part of dbAlm.

dbVas

One of the main tasks of ECVAM is to play a leading role at the European level in the independent evaluation of the relevance and reliability of tests for specific purposes, through research on advanced methods and new test development and validation.

Within this context, and to complement the information stored in dbAlm, it has been decided to centralise all information coming from formal validation studies and to create a system supporting the management of the whole validation process (the Intranet for study participants).

dbVas is therefore designed to manage all available information on international validation studies with reference to the different stages and overall status of a formal validation process, carried out under the auspices of ECVAM. The following data sectors are covered:

- Summary Records
- Test Protocols
- Test compounds and their Toxicological Profiles
- Test Results and Data Analysis
- Participating Organisations and Laboratories
- Follow-up Activities
- Background Documentation

ECVAM Workshop and Task Force Reports

ECVAM is required to ensure that it is well-informed about the state-of-the-art of non-animal test development and validation in relation to particular types of chemicals/products and potential toxic hazards.

ECVAM Workshops are therefore held to review the current status of various types of alternative tests and their potential uses, and to identify the best ways forward. ECVAM Task Forces focus on more tightly-defined targets.

SIS has established a bibliographic database to store all references analysed during the process of data collection for dbAlm, including those from formal validation studies. Because of their unique scientific relevance, all reports of the ECVAM Workshops and Task Forces have been included in SIS in the form of their conclusions and recommendations, and this will be shortly extended to cover their entire contents.

Origin of Information

Three main ways have been adopted for the definition of new information sheets to be stored in SIS: i) protocol definition, mainly locally at SIS; ii) study contracts for external data collection, and iii) centralisation of the information on validation studies through the Intranet. A fourth way involves informal collaborations on alternatives databases with specialised, mainly European, institutes, in order to exchange ideas and experiences and to avoid duplications.

In fact, the progress made on the collection of information for the database on alternative methods has been as a result of collaboration with organisations in Belgium, Germany, Italy and the UK. The major part of the documented information is compiled by experts, based on a careful analysis and synthesis of all relevant scientific information sources over the past twenty years.

However, the overall coordination, including guidance during data collection, the setting of criteria and critical assessment of the collected information before its storage in SIS (now done in an automatic matter), is carried out by the responsible ECVAM staff. Only in this way can quality assurance be guaranteed. In order to set up a uniform system in all its parts, as a basis for user friendliness during data retrieval, all documents for all data sectors are compiled in a uniform way, on the basis of previously defined formats and criteria for data content (Janusch and Aiello, 1998). A comprehensive classification scheme is used for their storage.

Furthermore, SIS maintains close collaborations with national centres experienced in the documentation of alternative methodologies for database purposes (e.g. Akademie für Tierschutz, D; Fund for the Replacement of Animals in Medical Experiments - FRAME, UK; and the Centre for Documentation and Evaluation of Alternative Methods to Animal Experiments - ZEBET, D).

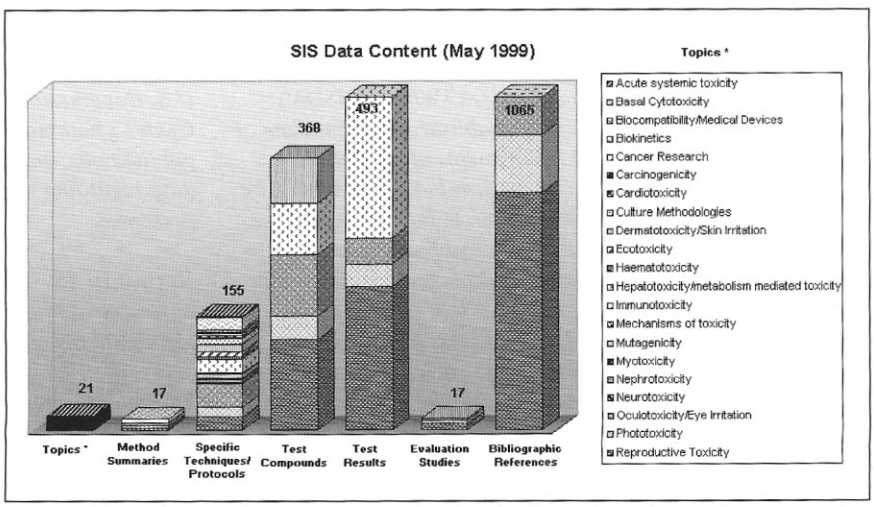
Customers

In establishing SIS, different user profiles have been taken into consideration, such as the Commission Services, industries, scientists and scientific organisations, as well as animal welfare organisations, or even the interested general public. Furthermore, SIS was also designed to eventually support the management of ECVAM's validation studies.

SIS in fact provides various kinds of information (e.g. method, test and study descriptions), as well as various levels of information (e.g. summary descriptions of methods and complete protocols with a step-by-step description for their performance; summary descriptions of validation studies, and the availability of related raw data). Furthermore, the decision to develop dbVas (fully integrated with the *INVITTOX* protocol collection) by using the Internet and Intranet has been made on the basis of a careful analysis of the whole validation process and the support needed by study participants.

Status

The present data content of SIS is outlined in Figure 1. *INVITTOX* protocols are now being updated at the ECVAM SIS and have become an integral part of dbAlm. Furthermore, a pilot study for dbAlm was successfully completed by the end of 1998. The study was focused on 9 priority topics in the area of *in vitro* toxicity testing for chemicals and formulations. Further data collection over the next two years will be guaranteed throughout an open call for tender



to be published in the Official Journal of the European Union. An inventory of *in vitro* pharmacotoxicology laboratories is at the planning stage.

Access

The final aim is certainly to make publicly available the full content of SIS, except for the confidential area of dbVas for security reasons during ongoing studies. Consequently, all the single sectors of SIS have been implemented as an interconnected database system. *INVITTOX* protocols, the major part of dbVas, as well as the ECVAM Workshop and Task Force Reports, have already been established by using the Internet. The complete version of dbAlm runs locally at ECVAM and will be transferred to the Internet at a later stage. Access to the first version of SIS with selected data sectors, will be provided through the universal communication media, Internet and Intranet, and is expected in early 2000. For the time being, protocols can be submitted by E-mail attachments. A CD-ROM version is also planned.

Planned Activities

With the establishment and maintenance of SIS containing factual (ready-to-use) information on alternatives, the problem of data retrieval in bibliographic databases has become evident. Furthermore, this problem has already been risen in various discussions and workshops (Janusch et al., 1997; Langley et al., 1999). Therefore, SIS is coordinating a Task Force on alternatives databases, in which the participating members are from FRAME (UK) and ZEBET (D), with the aim of improving access to articles on alternatives in traditional bibliographic databases covering various fields of sciences (e.g. MEDLINE, TOXLINE, EMBASE, BIOSIS, etc.). An a meeting together with the Head of the Thesaurus Section of the US National Library of Medicine, held in March 1999, the possibility of developing a thesaurus specifically on alternatives to animal experiments was discussed, and a pilot study has been initiated on selected digital texts. The thesaurus will be generated by using the novel "Bottom-Up approach", which differs from previous methods by being based on phrases which most commonly occur in documents. The study will explore the spectrum of information on alternatives and the utility of the resulting thesaurus will be verified by consultation with various end users. Preliminary results should appear within the next year.

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The animal welfare information center - a brief overview

Summary

The Animal Welfare Information Center (AWIC) of USDA's National Agricultural Library was established by a mandate of the U.S. Congress in the 1985 Amendments of the Animal Welfare Act. The Act regulates how warm-blooded animals are treated as they are in biomedical research, for toxicity testing, as specimens in higher education, or exhibited in circuses, zoos and aquaria. Large volume dog dealers also come under the Act.

In the 1985 Amendments, the U.S. Congress instructs the Center to provide information on topics such as: alternatives to painful procedures, anesthetics, analgesics, tranquilizers, improved methodologies, training materials for those who use animals, environmental enrichment for primates, and exercise for dogs. This overwiew of the AWIC program discusses the historical aspects of the Center, and will illustrate how the U.S. Congressional mandate is currently carried out via the Center's national and international activities, services, publications, and outreach to U.S. regulated community. Some data regarding the use of the Center's products and services is presented.

Keywords: 3R, animal welfare information center, national agricultural library, information services, publications, electronic resources, user statistics

Zusammenfassung: Das Animal Welfare Information Center - ein kurzer Überblick

Das Animal Welfare information Center (AWIC), das der National Agricultural Library der USDA angeschlossen ist,

wurde 1985 im Auftrag des U.S. Congress durch einen Zusatzartikel zum Animal Welfare Act eingerichtet. Dieses Gesetz regelt die Behandlung von Warmblütern, wenn sie in der medizinischen Forschung, für Toxizitätsprüfungen, für Lehrzwecke an höheren Schulen oder für Zirkusse, Zoos und Aquarien verwendet werden. Grosse Hundehändler fallen auch unter dieses Gesetz.
In Zusatzartikeln von 1985 wird das Information Center damit beauftragt, Informationen über folgende Sachgebiete zu beschaffen: Alternativen zu schmerzhaften Behandlungen, Betäubungs-, Beruhigungs- und Schmerzmittel, verbesserte Methodologien, Ausbildungsmaterial für den Umgang mit Tieren, enrichment für Primaten, Übungen für Hunde. Dieser Überblick über das AWIC Programm behandelt den historischen Aspekt des Centers und zeigt, wie das Center den Auftrag des Kongresses in nationalen und internationalen Aktivitäten, Dienstleistungen und Publikationen derzeit umsetzt. Einige Daten über die Benutzung der Angebote und Dienstleistungen des AWIC werden hier vorgestellt.

The animal welfare act

In the mid-nineteen eighties, laws such as *The Improved Standards for Laboratory Animals Act, Public Law 99-198*, were passed in the United States. This law, under the regulatory authority of the U.S. Department of Agriculture (USDA), was written by the U.S. Congress to further protect the health and welfare of animals that are used in biomedical research, testing, higher education or placed on exhibit. This amendment to the Animal Welfare Act (AWA) also mandates regular exercise for dogs and environmental enrichment for nonhuman primates.

The 1985 amendment to the AWA (first passed in 1996) mandates a number of important changes to the Act: It defines "humane care"; directs USDA to address exercise requirements for dogs, requires enriched environments for nonhuman primates; mandates that there be efforts on the part of the research community to minimise pain and distress; includes a requirement for the establishment of an Institutional Animal Care and Use Committee (IACUC); directs researchers to find and implement alternatives in their research that could reduce the numbers of animals used, replace animal use, and refine experimental procedures to reduce pain and distress; states that computer databases and other information resources are to be utilised to eliminate unnecessary duplicative research; and lastly and important from my point of view, it mandates that an information service be established at the National Agricultural Library to provide for the information needs of the regulated community. The "service" is the Animal Welfare Information Center or AWIC for short.

To begin with a few words about The National Agricultural Library (NAL): NAL is located on the USDA's Beltsville Agricultural Research Service in Beltsville, Maryland. It is one of 4 National Libraries. (The others are: the Library of Congress, the National Library of Medicine and the National Library of Education.) The Library, as a part of the USDA, was established by Abraham Lincoln in 1864. It has grown from a small collection of 1000 volumes to over 3.2 million items. NAL also generates the AGRICOLA database. The Library participates in a world-wide borrowing network called AGLINET. This network allows all members to exchange materials that are not found in their own countries library systems. Although one may not directly borrow materials, from NAL, the national library systems can request photocopies of articles.

Within this already established Library program, AWIC exists as one of several subject focused Information Centers. Each is quite different and has a different program that is responsible for different clientele. In regard to the AWIC program, the scope and subject focus of the Center is guided by the Congressional mandate that was quite specific. Basically, we are to provide the regulated community with 1) information pertinent to employee training, 2) preventing unintended duplication of animal experiments, 3) improved methods of experimentation that can reduce or replace animals use and/or 4) minimise pain and distress to animals. All the services, activities and publications are designed to meet these mandates. Here are more details regarding the animals covered, activities and services.

AWIC scope

Basically, the Center includes all vertebrates and invertebrates in research environments. However, at the discretion of the Secretary of U.S. Department of Agriculture, birds and members of the species *mus* and *rattus* as well as animals used for food and fiber research are not covered by the AWA. On the other side, the Department is being sued by U.S. animal protection groups to cover rats, mice and birds in biomedical research. As with many regulatory processes, the issue is mostly cost. There is currently not enough money for an increased level of inspector staffing for USDA/Animal and Plant Health Inspection Service's Animal Care Division. Congress is currently being pressured to increase the level of funding to cover the additional personnel costs needed to inspect rodent facilities. It is not clear when these issues will be resolved. In any event, the Center's species coverage is broader than is required in the AWA.

AWIC services

Basically, there are five important services that AWIC provides to patrons: 1) reference, 2) referrals, 3) training, 4) subject specific publications, and 5) outreach activities. On each of these services is given a more complete picture of the AWIC program.

1) Reference

A high percentage of AWIC resources are utilised for reference services. Although the Center was established to serve the regulated community, the program is a part of the National Agricultural Library, therefore we are obligated to respond to any U.S. citizen, the non-regulated community, with a question regarding animals.

The non-regulated community queries are usually regarding pets and farm animals. Questions may be as diverse as: breed specific dog behaviours; how to take care of hedgehogs; information on the housing and husbandry of emus and ostriches as production animals; the pros and cons of hip replacement for a pet dog; etc. Since this community of users is not the focus of the Center, we provide less in-depth responses to these questions than we do for the biomedical and other sectors of the regulated patrons.

For the regulated community, we have a much greater responsibility to provide comprehensive reference service. The service routinely includes multiple database searches with complicated search algorithms. Such searches are often done to help a researcher meet the information requirements of the AWA regarding unnecessarily duplicative research, addressing the 3Rs of Russell and Burch (1), improved methodologies and/or pain control, housing and husbandry of the model animal, and issues regarding IACUC decisions. Some examples of queries from this group might include an alternatives search to the use of anaesthetized dogs in surgical instruction at a veterinary or medical school; alternatives for a protocol describing a thoracotomy in dogs; the best method of euthanasia when brain tissue is to be harvested; or enrichment strategies for primates housed in a zoo setting. Most of the requests come from those in biomedical research or toxicology testing.

2) Referrals

Referrals are often requested by patrons, or the staff recommends that a patron contact an expert in the field for further information. The referral may be to a person, organization, someone trying to solve a similar problem. Non-traditional resources such as unique databases and web sites may also be recommended as additional resources.

3) Training

A very important activity of the AWIC program is providing researchers with training for conducting and evaluating the "alternative search". Since it was obvious that AWIC does not have the resources to provide all researchers in the U.S. with the complicated searches for alternatives, the staff developed a workshop on conducting the alternative search. There are several versions of the workshop that can be given at different venues. The most comprehensive is a formal workshop "Meeting the Information Requirements of the Animal Welfare Act". It is a 1 day workshop that is conducted by all the staff in the NAL facility. The subjects covered include instructional sections on the history of animals protection in the U.S.; the information requirements of the AWA; the concepts of the 3Rs by Russell and Burch (1); resources for alternative information; how to do an alternatives search; and evaluating the search.

The full version of the workshop includes hands-on sessions at the computer with access to multiple databases on the DIALOG system. Two and four hour versions of the workshop are conducted in a variety of venues. In such cases, the attendees receive information about the 3Rs concepts and examples of searches are demonstrated, but there is not hands-on experience by the attendees. The host institution is responsible for paying the costs for travel, lodging and meals for the staff who will be conducting the workshop. It is also expected that the host institution copy any support materials, supply projection equipment and computer hook-ups, etc.

To date, the 1,5 day workshop is conducted at NAL three times/year. Depending on the number of requesters, the abbreviated versions may be conducted five to 10 times/year at pharmaceutical companies, academic institutions, and regional and/or national meetings of professional and military organisations. Attendees at all workshops include researchers, IACUC members, attending veterinarians, facility managers, information providers, USDA, Animal Care inspectors, and animal protections folks. The mix of people with different perspectives usually leads to interesting discussions.

4) Publications

Publications of various types have been a very important part of the AWIC program since the early years. In order to meet the demands for a wide variety of information, unique non-traditional types of publications have been developed. A quick overview shows the more important series that we publish.

- Quick Bibliographies (QB's) are bibliographies of 200-300 references that are produced from information in the AGRICOLA database. In many cases, the topics are related to production animal topics or other subjects where this database has extensive coverage of U.S. materials. Examples include anaesthesia of animals; and housing, husbandry, and welfare of the various livestock animals.
- Special Reference Briefs (SRB's) are also bibliographies, but are more comprehensive. They are produced from multiple sources both electronic and paper. They are reviewed by scientists knowledgeable in field and include an introduction to the topic. Titles have included Animal Euthanasia, Zoonotic Diseases, and raising of ratites.
- AWIC Series have been produced for special needs of the regulated clientele. They are usually comprehensive listings of resources. To date, there are listings of selected data bases, web sites, and a listing of audiovisuals in the NAL collection.
- The AWIC Resource Series includes documents that are also topical but very comprehensive. They are largely bibliographic, but contain introductory topical information, listings of information resources, relevant organisations, and companies that either provide products or services. They are often collaborative efforts with authors from institutions or organisations both in the U.S. and abroad.
- There are a variety of miscellaneous publications that do not fit into a series they include conference proceedings, manuals, guides, etc.
- The Animal Welfare Information Center Bulletin is a free newsletter that consists of several major articles on a topic relevant to the AWA regulated community, a column on major legislation introduced into the U.S. Congress, sources of grants, etc. Currently, it has a subscription list of about 7,000 and includes individuals and organisations in over 30 foreign countries. If you are interested in receiving the *Bulletin*, feel free to contact AWIC to get added to the mailing list or read the electronic version on the AWIC web site.

AWIC on the World Wide Web [http://www.nal.usda.gov/awic]. As with many organizations, AWIC has developed a web site of electronically available information. Although many of the AWIC publication both current and out-of-print are available on the NAL/AWIC web site, the site also contains other types of information such as full text government documents, other relevant documents and important links to many organizations and resources. In addition, where URL's are mentioned in documents, efforts are made to keep live links. Currently, the site is organised into 12 sections: Legislation, Regulations and Policies; AWIC Newsletters; Publications; News and Information; Databases, Alternatives, Farm Animals, NAL, APHIS Animal Care, etc.

In the last 7 months, we have statistical data that show that 6,178,143 Kbytes have been downloaded from the site. If you are looking for information on alternatives, consider adding AWIC's address to your list of favourites.

Activities

5a) Outreach

Since AWIC is a Federally mandated program that is to provide services to those regulated under the AWA, we spend time and effort making sure that the potential users of AWIC services know what we have to offer. Therefore, we engage in the following outreach activities: presentations on such topics as the products and services, alternatives to research and the alternatives search. On occasion staff author and deliver papers or presentations on related subjects. We also exhibit at a variety of meetings - professional, professional society annual meetings, workshops, governmental meetings, etc.

5b) Miscellaneous activities

In addition to all the routine services and activities mentioned above, the Center is often involved in special projects. In the past these have included an active grants/cooperative agreements program to produce only information products, participating on national and international conference planning committees, agency representatives on committees and at workshops, USDA inter-agency committees, as members of a Federal IACUC, production of training programs sponsored by multiple partners, etc.

We are always interested in working with national or international partners on projects that produce information related products and/or information that will help the research community improve the status and environment of research animals.

Table 1: The AWIC fiscal year 1998 statistics

Reference requests processed Publications distributed	2,211
AWIC produced documents	33,374 items
Documents from other organizations	2,209 items
Outreach and training	
47 outreach events	3,493 individuals
7 training events	166 individuals
The users usually fall into the following groups:	
Disciplines directly involved with the animals	49.4%
Educators/students	10.0%
Government personnel	5.1%
Information/informatics	4.0%
Other disciplines	16.4%
Patrons had the following general affiliations:	
Biomedical research	40.9%
Educational institution	18.6%
General public	15.6%
Government office	11.1%
Zoos/aquaria/circusses	4.1%
For profit and non profit organizations	3.7%
Advocacy groups	3.3%
Libraries	2.6%
Although the AWIC has more that seven topics that may be assigned to a query, we have collapsed the topics into the following seven general topical areas:	
AWIC/NAL related	23.6%
Alternatives - the 3Rs	18.0%
Animal care	10.0%
Techniques/enrichment	8.2%
Laws and regulations	6.7%
Disease/physiology	6.6%
Databases/database searching	6.1%

User statistics

The AWIC program has matured into a successful, multi-faceted program since 1986. There is still much to do, and we do have a several activities and publications that we are currently working on. There will be several more publications on topics such as IACUC's, euthanasia, anesthesia, opossums as a biomedical model, and a large information resource on swine. We are also collaborating with several groups on an interactive web and CD ROM based training module.

To keep aware of AWIC activities and information products, the address mentioned below should be contacted.

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Experience in Sweden

Summary

In Sweden animal experiments for research and testing purposes are assessed from an ethical point of view by regional animal care committees. According to the legislation the committees shall consider whether the experiment could be carried out in a less painful manner and, if so, make the necessary changes in the protocol. Existing alternative methods should always be used. The authority responsible for the committees is the Swedish Board for Laboratory Animals (CFN). One of its tasks is to reduce the number of animals used for experiments, e.g. by promoting alternative methods. The CFN issues recommendations to the ethical committees on the treatment of certain animal experiment proposals, and has set up an advisory board to assist the committees. Unfortunately, there is no follow-up with regard to the recommendations, and the advisory board is constructed in such a way that nobody seeks its advice.

Looking back at 20 years of animal care committees it is evident that the support given so far by the authority is insufficient. However, there are many ways that this situation could be improved.

Keywords: 3R, animal care committees, animal ethical committees, Sweden

Zusammenfassung: Erfahrungen aus schwedischer Sicht
In Schweden werden Tierversuche für Forschung und Tests durch regionale Tierschutzkommissionen nach ethischen Gesichtspunkten begutachtet. Die Gesetzgebung verpflichtet die Kommissionen zu prüfen, ob ein Experiment auf eine weniger schmerzhafte Weise durchgeführt werden könnte und, wenn dies der Fall ist, die notwendigen Änderungen im Protokoll zu vermerken. Bereits existierende Alternativmethoden sollten immer angewandt werden.
Die für diese Kommissionen verantwortliche Behörde ist das Schwedische Amt für Labortiere (CFN). Eine der Aufgaben dieser Behörde ist es, die Zahl der Versuchstiere zu reduzieren, z.B. durch die Förderung von Alternativmethoden. Das CFN veröffentlicht für die Ethik-Kommissionen Empfehlungen für die Behandlung bestimmter Versuchsanträge und hat ein Beratungsgremium für die Kommissionsmitglieder eingesetzt. Leider werden die Empfehlungen nicht auf dem laufenden gehalten, und das Gremium ist so zusammengesetzt, dass niemand seine Dienste beansprucht.
Im Rückblick auf 20 Jahre Kommissionsarbeit muss festgehalten werden, dass die Unterstützung der Behörde unzureichend ist. Es stehen jedoch viele Wege offen, um diese Situation zu verbessern.

Preview of all planned animal experiments

In Sweden practically all animal experiments for research and testing purposes are subject to review from an ethical point of view by regional animal ethical committees. This system was established in 1979, that is now 20 years ago. Sweden was the first country in the world to set up animal ethical committees on a non-centralised, open level. Each committee is chaired by a person from the legal profession. Of the – normally twelve – members, one half are researchers. In this context, this refers to persons who perform animal experiments, and to staff who work with experimental animals. The other half are laymen having varying professions. Some of the laymen represent the general public and are proposed by the municipality. Two – at the most – of the laymen represent animal welfare organisations.

The committees shall consider, among other things, whether the experiments can be carried out in a manner less painful to the animals, or whether the experiment could be replaced with an alternative method. This task means that the committees should have, or seek, the necessary knowledge to assess a great variety of procedures, and also know about established or possible alternative methods.

In other words:

- a committee should identify painful and very painful procedures and act accordingly;
- it should identify procedures that will cause the animal very great or undue anxiety and act accordingly;
- it should know about alternative methods and act accordingly.

In order to carry out these tasks in a satisfying way, the animal ethical committees need

- information on the current state of knowledge on laboratory animal science (e.g., on pain and anxiety in laboratory animals; how different strains react to various anaesthetic and analgesic drugs; how they react to different kinds of enrichment, etc.)
- information on existing alternatives to animal experiments.

The Swedish Board for Laboratory Animals

The authority responsible for the animal ethical committees in Sweden is the Swedish Board for Laboratory Animals (CFN). The CFN was established at the same time as the ethical committees, and was made responsible for the committees in 1981. To be able to live up to its responsibility, the authority ought to have a grasp of the animals experimentation and alternatives situation. For instance, it ought to have an idea of how many and what kind of really painful experiments that are performed; and also, what experiments are accepted by the animal ethical committees, in spite of existing *in vitro* alternatives. The applications should be on file at the CFN, thus enabling the CFN to prepare more detailed statistics of various kind of experiments.

The Swedish Parliament has endorsed the view put forward in the EU 5th Environmental Programme that the number of animal experiments should be reduced by 50%. However, it seems difficult to work out a plan to reach this goal if the responsible authority does not know in detail what kind of animal experiments that are performed.

How does the CFN meet the needs of the animal ethical committees?

Information disseminated by the CFN:

The CFN Series

The CFN issues booklets, known as The CFN Series, on various matters relevant to the use of laboratory animals. Some of these are reports from conferences organised by the CFN, e.g. the symposium The Effects of Toxic Substances on the Gene Expression and Intracellular Signalling, which was held in September 1997. Some issues in the series contain the annual statistics on the use of laboratory animals. And finally, there are some issues that touch on matters of more immediate concern for the committees, like identifying and treating pain in laboratory animals. Unfortunately, these issues of more hands-on information are few and far between.

The CFN Recommendations

Apart from the publications in the CFN Series which treat matters in a general way, the CFN also issues recommendations on how the committees should treat specific matters. For instance, there is a recommendation from 1990, about the production of monoclonal antibodies, stating that in most cases the monoclonal antibodies can be propagated *in vitro*. If a researcher claims that it cannot be done without the use of animals (the ascites method), it is the responsibility of the committee to assess whether this is really the case. However, during the past seven years, no application to produce monoclonal antibodies with the ascites method has been rejected by the committees, although the justifications given by the researchers have been scanty or practically non-existent. This example illustrates that the ethic com-

mittees are in need of hands-on information of existing alternatives to animal experiments. This is particularly important, since one of the tasks of the committees is to give advice to the researchers. The CFN has not reacted to this situation, perhaps due to the fact that the animal experiment applications are not filed by the CFN but by each regional committee.

In contrast, the Swedish Society Against Painful Experiments on Animals has filed all applications since 1979.

The CFN Advisory Panel

In 1996 the CFN established a panel of experts in various matters, e.g., transgenic animals, ethology, anaesthetics and analgesics, etc., with the purpose of providing the ethical committees with information. However, at the same time the committees were told that the panel should not be consulted by any single member of the committee, but only by the committees' own expert on the matter. As might be expected, it seems that the committees' own experts do not feel the need to consult others; consequently, the panel has hardly been of any use to the committees. Apart from the restrictions in contacting the advisory panel, many members of the committees do not even know about it, possibly due to the fact that new members are not informed about its existence.

Future development of information services

To date, the information service provided by the CFN to the animal ethical committees has been far from satisfactory. The Swedish Society Against Painful Experiments on Animals has repeatedly criticised the lack of initiative on the part of the CFN and put forward suggestions on how the situation could be remedied. The concerns of the Swedish Society Against Painful Experiments have also been voiced during the years by a number of Members of Parliament. As a result, the government ordered an investigation to be made which in its turn has led to a memorandum, issued by the CFN and the Swedish Board of Agriculture (the Swedish authority with responsibility for all animals, domestic or otherwise kept, including some areas of animal experimentation).

The memorandum identifies a number of areas where action will be taken to

- improve the situation, among these
- a database of all animal experiment applications assessed by the animal ethical committees.

It is to be hoped that this database will enable the CFN to assess the decisions of the ethical committees, e.g. find the cases where simple measures would have enriched the lives of laboratory animals, the cases where alternatives would have replaced the use of laboratory animals, and the cases where the use of adequate anaesthesia and analgesics would have spared the animals unnecessary pain.

- employment of a person to serve the animal ethical committees with information about alternatives and other matters relating to the use of laboratory animals.

If this person is easily accessible by single members of the ethical committees, as well as competent in information retrieval, from databases or otherwise, and dissemination of information about alternatives, laboratory animal science etc – then he/she will be of great help to the Swedish ethical committees.

- the possibility to strictly regulate or even ban single animal experiment procedures such as the LD_{50} and LC_{50} tests, the ascites method in the production of monoclonal antibodies.
- the possibility of a clear cut ban on animal experiments in high school.

The Swedish reviewing system has been in existence twice as long as ZEBET, but has not even achieved half of what ZEBET has as regards information services to the animal ethical committees. If the planned changes come true it will mean some steps towards a better situation.

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Empowering the End User - the UK Perspective

Summary

Recent changes to the UK Animals (Scientific Procedures) Act have increased the emphasis on the requirement for project licence applicants to be aware of all practical possibilities for implementing the 3Rs. Applicants will be expected to document all steps taken to obtain this information, including literature searches and other forms of enquiry. The simultaneous establishment of a local ethical review process within every designated establishment has likewise increased the need for such information. In the latter case, this information may be required by ethical committee members who are outside the proposed field of study, or are not scientifically qualified. It is essential to assist all these potential enquirers to identify relevant information, especially when no adequate professional information backup is available. The different aspects of such a process will be described.

Keywords: 3R, information retrieval, end-user searching, search techniques

Zusammenfassung: Unterstützung für den End-Verbraucher - die englische Perspektive

Die kürzlich erfolgten Änderungen im englischen Tierschutzgesetz (UK Animal ((Scientific Procedures)) Act) *haben den Druck auf die Antragsteller für Tierversuche verstärkt, alle praktischen Möglichkeiten einer Anwendung des 3R-Prinzips zu berücksichtigen. Es wird von den Antragstellern erwartet, dass sie alle Schritte dokumentieren, die sie zum Erhalt der entsprechenden Informationen, einschliesslich Literaturrecherchen und andere Nachforschungen, unternommen haben. Die gleichzeitig erfolgte Einrichtung lokaler ethischer Prüfprozess innerhalb jeder designierten Einrichtung hat den Bedarf nach solchen Informationen stark erhöht. Im letzteren Fall können die Informationen von Mitgliedern des Ethikkomitees angefordert werden, die nicht wissenschaftlich qualifiziert sind und von ausserhalb des zur Debatte stehenden Fachgebietes kommen. Es ist wichtig, all den potentiellen Anfragenden bei der Identifizierung relevanter Informationen zu helfen, besonders wenn keine genügende Fachinformation erreichbar ist. Die verschiedenen Aspekte eines solchen Prozesses werden hier beschrieben.*

3Rs information - a current topic

The dissemination of information on the 3Rs is a topic that has become very current in the UK due to two recent changes in the workings of the *Animals (Scientific Procedures) Act 1986*. Firstly, some of the wording has been revised:

„The Secretary of State shall not grant a project licence unless he is satisfied (a) that the purpose of the programme to be specified in the licence cannot be achieved satisfactorily by any other reasonably practical method not entailing the use of protected animals; and (b) that the regulated procedures to be used are those which use the minimum number of animals, involve animals of the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress or lasting harm, and are most likely to produce satisfactory results."

As a result, the Act now states very clearly that any proposals to use laboratory animals must show that due consideration has been given to the 3Rs. One of the ways in which this can be demonstrated is through a documentation of the steps which project licence applicants have taken in order to become informed of these possibilities. This documentation may include a description of the literature searches that have been conducted, together with a listing of databases interrogated and the search terms employed. Other routes of enquiry may also be described. These could include searches on the Web using Internet search engines, posting of queries to newsgroups and mailing lists and the consultation of the list archives, as well as discussions with colleagues.

The second change is the introduction of the requirement that from April 1st of this year, every designated establishment must have a local Ethical Review Process in place. This consists of a committee which can include both scientists and non-scientists, people from inside the establishment as well as people from outside and which will scrutinise each proposal before an application is made to the Home Office for a project licence. The committee will look at the possibilities for implementing the 3Rs as well as the cost-benefit implications of proposed animal studies. The members of a local Ethical Review Process cannot be expected to have the same detailed knowledge of the field as the proposer of a study. They will therefore require information about the background leading up to the proposal, as well as a general awareness of the 3Rs. If lay members are included, they will not have access to the informal network of communications that exists between scientists. Thus, the establishment of the local Ethical Review Process has opened up a further requirement for information on the 3Rs.

Difficulties of finding 3Rs information

Any attempt to collect information on the 3Rs is beset by difficulties. The most significant difficulty arises from the fact that the 3Rs are concerned with the methodology of a study: the systems used and the procedures employed. On the other hand, the reporting of scientific activity that takes place within the scientific literature tends generally to be more focused on the hypothesis being tested and on a discussion of the results obtained. As a result, even if the major bibliographical databases were to expand their indexing strategies so as to include concepts relating to the 3Rs, many relevant papers would still not get indexed from this facet because the 3Rs would not be sufficiently emphasised in the original article to make this recognisable to the indexers of the database.

One partial solution to this problem might be to have authors use 3Rs-related keywords, whenever applicable, in their abstracts. These words could be used as search terms to extract the abstracts from a database. For this to be at all feasible, it is necessary to draw up a manageable list of agreed keywords and to obtain the cooperation of authors in using them. Ideally, journal editors could also cooperate by promoting the use of the keywords, for example, by including a list of 3Rs keywords in the instructions to authors wishing to publish in their journals. Whether such a radical shift in perspective is feasible across the whole spectrum of scientific authorship is, however, highly questionable.

A second difficulty can arise from the fact that a replacement, refinement or reduction alternative developed in one field of study could well have useful application in another field. The problem is how to identify these possibilities across the disciplines when each discipline will have its own set of core literature which may not be known to the scientist putting together a project proposal.

The net result of these difficulties is to make scientists often feel that the requirement to search of 3Rs information is an unwanted burden which delays the whole process of licence application. This was the viewpoint stated by the report on regulatory burden which was recently produced by the US NIH (1999) and which drew the somewhat arrogant conclusion that „The scientific community contends that this rarely will provide an understanding of the thought given by the scientist to the examination of alternatives and the best methodological approach to the hypothesis being tested." In effect, the report went on to recommend that searching for information on the 3Rs should not remain a legal requirement. Instead, the local IACUC should decide on a case by case basis whether a formal, documented literature search was required or not. In effect, the decision whether or not a search was required would be made by IACUC members who themselves might not be fully conversant with what information might be available.

Approaches to a solution

It is obvious that any attempt to promote the development, use and full acceptance of 3Rs methodologies must therefore include, as an integral part of the strategy, an effort to surmount some of these difficulties.

The first, and perhaps most important, step is to convince scientists that being informed about the 3Rs can work to their advantage by enhancing the quality of scientific research. Thus, the use of *in vitro* systems, perhaps as part of a preliminary study, can assist in bringing about a clearer mechanistic understanding of the processes under investigation, because it is possible to isolate them from systemic influences. The use of reduction strategies, can lead to improved experimental design and increased precision of data, while attention to refinement, by maximising the well-being of the animals, can minimise the variance that may arise due to the influence of stress. Moreover, if the search for alternatives is broadened into other fields, it can introduce a valuable cross-fertilisation between the methodologies of different disciplines.

Having made this point, it is then logical to argue that a search for information on the 3Rs should be an integral part of any literature search and other enquiries that are pursued at the initial stages of project conception and planning. By making consideration of the 3Rs an automatic and integral part of the early stages of this process, the investigator should not feel subject to any pressure in this respect at the time of applying for a licence since the work will already have been done. From the viewpoint of animal welfare, it is more likely that reduction, refinement and replacement will be implemented to the maximum if they are considered at an early stage rather than at a time when the structure of the project might already have become fixed.

However, it is not enough to try to convince scientists of the value of searching for 3Rs information. Some assistance in this matter is also required. It is necessary to show where this information can be found and to describe how best to search for it.

Although a number of specialised information resources do exist, none of them is fully comprehensive. For example, INVITTOX was set up by FRAME in response to the perceived difficulties of finding detailed methodological information on alternative methods. While it has been recognised as performing a useful function, it is limited to the area of *in vitro* toxicity testing. Bibliographical databases on alternatives, such as the one set up by the Akademie für Tierschutz will necessarily be limited by the literature which is available locally to the database compilers and by the human resources which are available to scan this literature. It is the opinion of this author that a fully comprehensive database encompassing reduction, refinement and replacement in all fields of biology and medicine is both logically and logistically impossible. To achieve such a database would require the re-indexing of the complete world biomedical literature from a 3Rs viewpoint. Even were that to happen, not all alternative approaches would be identified as long as the tendency to cut down on methodological details in scientific papers continues.

Therefore, it is necessary to educate the end user not only about the specialised resources that exist, but also about how to search the scientific literature in general and how to pursue other lines of enquiry. To this end, the author has prepared an information resource which will shortly be placed on the FRAME web site where it will be available to researchers, members of the local Ethical Review Process and all other interested parties. Elements of the same approach are also used in presentations at workshops on alternatives and for the Module 5 training which is mandatory for all project licence applicants in the UK.

Topics covered include the overall approach to searching, namely, the definition of a search strategy, the selection of search terms, how to build these terms into a search profile using search operators and how to broaden or narrow a search. The use of the Internet is also discussed, for example, how to identify and use mailing lists and newsgroups, how to use various search engines and meta-searchers. While the examples used relate to the 3Rs, the material does provide tips on how to approach any kind of query. It is the author's experience, that this added value, which is of general use, does help to overcome the resistance some scientists may show when asked to listen to presentations on alternatives.

Why focus on the end user?

It may be argued that too much emphasis has been placed on the end user. Certainly, the ideal procedure is for an information search to be carried out as a process of dialogue between the end user and an experienced information professional who has the necessary knowledge of sources and techniques. However, many scientists, especially those in academia do not have ready access to the individual attention of information professionals. When finances are tight, libraries and information services are often in the first line of any budget cutting exercise and may have to limit themselves in the services they provide. Secondly, many scientists are resistant to the idea that someone else might be better qualified to satisfy their information needs. While it is obviously necessary to counter this attitude, and to raise the status and profile of the information profession, it is also necessary to be pragmatic and to find solutions within the situation as it exists today.

The establishment of the Ethical Review Process will with no doubt increase the number of people seeking information on the 3Rs. However, there is no guarantee that there will be a simultaneous increase in the resources available to satisfy this need. Unlike the case of ZEBET, the UK has no national centre to which these queries can be addressed. While FRAME does respond to queries, it does not have the financial or human resources to carry out the sort of detailed searches which might be required in some cases. Giving the end user the tools with which to carry out a search is an attempt to find a partial answer to the problem. While this is by no means a satisfactory solution, the hope is that it will contribute in some measure to promoting awareness of the 3Rs among scientists and those involved in the Ethical Review Process both in the UK and elsewhere.

Reference

NIH (1999). *NIH initiative to reduce regulatory burden: identification of issues and potential solutions.* http://www.nih.gov/grants/policy/regulatory-burden/

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Selected Posters

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Organotypic hippocampal slice cultures as in vitro model for testing neuroprotective drugs in preclinical stroke research

Abstract

In vivo *models of ischemia cause substantial pain and anxiety to the animals involved.* In vitro *models have been developed in order to overcome these problems. Organotypic brain slice cultures combine the advantages of preservation of native neuronal circuitry as it is found* in vivo *with strict control over environmental conditions and easy accessibility to manipulations. We have developed organotypic hippocampal slice cultures as an* in vitro *model for the investigation of drugs which may protect neurones from ischemic damage.*
Slice cultures prepared from neonate rats were maintained on membrane filter inserts at the interface between tissue culture medium and atmosphere.
Ischemia was simulated by combined oxygen/glucose deprivation. Functional neuronal damage was estimated by electrophysiological recordings of field potentials. Neuronal cell death was measured by propidium iodide uptake 24 h after the insult. Pharmacological validation was achieved by testing the effects of cytoprotective compounds with different effector mechanisms.
Results obtained by measuring functional neuronal damage and neuronal cell death were highly comparable. Inhibitors of NMDA-receptors, Na^+ channels, Na^+/H^+, and Na^+/Ca^{2+} exchangers had protective effects, but not inhibitors of AMPA/kainate receptors, metabotropic glutamate receptors, and Ca^{2+} channels or free radical scavengers. About four different conditions can be tested using slice cultures from a single animal, which means a reduction in animal consumption by 75%.
Taken together, organotypic slice cultures provide an experimental in vitro *system that is well suited to complement* in vivo *preparations in studying long-term pathophysiological processes of neurodegenerative diseases. Bearing in mind that slice cultures prepared from neonate rats may not represent the situation found in the adult CNS and that it is not possible at the moment to cultivate hippocampal slices from adult rats, we have started to develop the preparation of organotypic hippocampal cultures from juvenile rats. These may represent a situation that resembles the adult status more closely.*
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***In vitro* adhesion and invasion of epithelial cell lines by Salmonella strains of different epidemiological origin**

Abstract

Salmonellas are widespread pathogens of humans and animals. They carry a variety of virulence factors including adhesion and invasion capability, whose activation in the infected host will determine their pathogenic abilities. However, only little is known about the involvement and the importance of such factors for the pathogenesis of salmonellosis under field conditions.
In the present study we have investigated the adhesion and invasion abilities of Salmonella strains of different epidemiological origin using different permanent epithelial cells (IEC-6, VERO) and a primary cell line from the small intestine of a calf foetus (pKD). The Salmonella strains were grouped according to their epidemiological origin:
Group 1: strains isolated from organs of calves which had died of salmonellosis
Group 2: strains isolated from rectal swabs of healthy animals during diagnostic investigations of herds
Group 3: strains isolated from organs of animals which had died or were killed because of other diseases than salmonellosis
Strains of different groups showed no differences in their ability to adhere to the cells tested. Significant differences were found for the invasion ability. Strains isolated from organs of calves suffering from salmonellosis showed a significant higher invasiveness for permanent cell lines and a noticeable higher invasiveness for pKD cells than strains of the other two groups.
The higher invasiveness, measured in an in vitro *cell culture model, for salmonellas isolated from organs of calves which had died from salmonellosis point out that invasion is an essential step required for salmonellosis under field conditions, too.*
The in vitro *determination of the invasion capacity of salmonella strains gives an indication of differentiation between virulent and less virulent strains and is also a possible way to check mutant strains for their virulence without animal experiments. The permanent cell lines IEC-6 and VERO were recommended for the investigation of invasion properties of salmonella strains.*

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Cytotoxicity and membrane toxicity of usual surfactants

Abstract

Surfactants are known for provoking local irritations on skin, mucosa and eye. The unwanted side effects of surfactants are established probably in their interface active properties. The extent of these local irritations depends from the kind of the surfactant, from the concentration, from the exposition time as well as the individual sensibility of the concerning person.

To evaluate their compatibility for skin and mucosa 18 usual surfactants were investigated with two in vitro *test systems. The cytotoxicity was determined with the XTT tetrazolium reduction assay EZ4U. This modified XTT test measures the ability of living cells to reduce a colorless tetrazolium salt to an orange water-soluble formazan derivative by mitochondrial dehydrogenases. The half maximum cytotoxic concentration (CC_{50}) was calculated by regression analysis from dose response curves. To ascertain the membrane toxicity the arachidonic acid release test (AART) was used. [^{3}H]arachidonic acid ([^{3}H]AA) is rapidly incorporated into cell membrane phospholipids. Due to membrane disintegration or enzymatic catalysis, it is released into the cell culture medium and can be measured by scintillation technique. As a parameter for the membrane toxicity the minimal toxic concentration (MTK) was ascertained, which describes the lowest concentration causing a significant [^{3}H]arachidonic acid release in comparison to untreated cells.*

Between the various kind of surfactants are great differences concerning their toxicity. In comparison with well-known surfactants the 18 tested substances were classified in severe irritant, mild to moderate irritant and non- or slight irritant.

Seven surfactants were classified as strong irritating, among them the standard irritant sodium dodecyl sulfate (SDS). After 1 h exposition time these surfactants were cytotoxic and membrane toxic below 10 µg/ml. The cationic surfactant benzalkonium chloride, two polyethylene glycol alcohol ethers Brij®78 and Cremophor® A25, the anionic surfactants sodium cetearyl sulfate, sodium dodecyl sulfate and sodium laureth sulfate and further the amphoteric surfactant cocamidopropyl betaine were classified as severe irritant. The standard irritant SDS was less toxic than the other six strong irritating surfactants.

Three alkylpoly glucosides (lauryl glucoside, undecyl glucoside, decyl glucoside) and the octylphenoxy polyethylene glycol Triton® X-100 were classified as mild to moderate irritant. Their cytotoxicity and membrane toxicity parameters were found at a concentration between 10 and 100 µg/ml.

Seven tested surfactants - Polysorbate 80, Soybean lecithin, three further alkylpoly glucosides (nonyl glucoside, octcyl glucoside, hexyl glucoside), Cremophor® EL and Poloxamer® 188 - were classified as non or slight irritant. Their in vitro *toxicity was found above 100µg/ml.*

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New molecular bioassays *in vitro* for the estimation of the teratogenic potency of valproic acid-derivatives.

Abstract

Therapy with the antiepileptic drug valproic acid (2-propylpentanoic acid, VPA) during early pregnancy can cause similar teratogenic effects (neural tube defects) in human and mice. In this study a new molecular bioassay is presented using following endpoints: differentiation of F9 teratocarcinoma cells, altered cell morphology, induction of possible targed genes, and the induction of viral RSV-promoter. The induction of a transiently transfected viral (RSV) promoter driven luciferase gene by VPA was used to screen a set of VPA-derivatives. Structure-activity investigations showed: the longer the aliphatic side chain the more the induction of the RSV-reporter gene. The specific induction was stereoselective. The teratogenic enantiomer S-4-yn-VPA (2-propyl-4-pentynoic acid) induced the RSV-driven reporter gene while the non-teratogenic R-4-yn-VPA does not. The relative potency of the tested compounds was (at 300 µM tested): heptyl-4-yn-VPA (2-heptyl-4-pentynoic acid) > hexyl-4-yn-VPA > pentyl-4-yn-VPA > S-4-yn-VPA > rac-4-yn-VPA > butyl-4-yn-VPA > 4-en-VPA (2-propyl-4-pentenoic acid) > VPA. Heptyl-4-yn-VPA was the most potent teratogen in vitro *and* in vivo.

Non-teratogenic VPA-derivatives like R-4-yn-VPA and 2-en-VPA (2-propyl-2-pentenoic acid) were ineffective in this system. Thus, the teratogenic effect of VPA and VPA-derivatives in the mouse correlated with the specific induction of the viral RSV-promoter controlled reporter in F9-cells. Acid compounds such as fatty acids are known to interact with peroxisome proliferator-activated receptors (PPARs). To test structure-acitivity relationships by VPA or its derivatives we used CHO cells stability expressing hybrid proteins of the ligand-binding domain of either of the PPARs. The teratogen VPA and the

teratogenic derivatives of VPA activated the PPAR-d construct in a very specific structure- and stereoselective way which correlated well with the activities in the reporter gene assay (bioassay) and those in vivo. *No such correlation was found with respect to activation of PPAR-a or PPAR-g. These structure-activity relationships indicate that PPAR-d may be a potential mediator of VPA-induced differentiation of F9 cells and may possibly be involved in the mechanism of teratogenicity of VPA* in vivo. *Furthermore two bioassays were designed with clearly defined endpoints, amenable to automation and screening of great number of compounds.*

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A tiered *in vitro/in vivo* testing strategy to classify severely eye irritating chemicals using two alternatives to the Draize eye test, the HET-CAM test and the 3T3 NRU cytotoxicity
Prevalidation and validation

From 1988-1992 a validation study was conducted in Germany on two *in vitro* alternatives to the Draize eye test in order to replace the Draize eye test for testing severely eye irritating chemicals. In an earlier test development project funded by the German Department of Research and Technology (BMBF) at Henkel and University of Münster, the HET-CAM test and the 3T3 cell NRU cytotoxicity test (NRU CT test) had shown promising results. The formal validation study co-ordinated by ZEBET and funded by the BMBF was conducted in two phases: phase I, prevalidation stage and ring trial (1988-1990), phase II, data base development (1991-1992). During prevalidation the two *in vitro* assays were established in 13 laboratories, standard protocols were developed including PC based software programs for data recording and 34 chemicals were selected for the ring trial. In the one year ring trial the two *in vitro* methods were validated with 34 coded chemicals under blind conditions in 13 laboratories in order to evaluate the reproducibility of the two assays within and among laboratories. To avoid Draize eye tests, the 34 existing chemicals backed by high quality literature data were chosen as test chemicals. In the ring trial on the one hand the NRU CT test showed a much better reproducibility than the HET-CAM test. On the other hand the HET-CAM test allowed a significantly better classification of eye irritation properties than the cytotoxicity test when compared to the Draize eye test. The HET-CAM test seemed to hold promise particularly for classifying severely eye irritating chemicals, which have to be labelled R-41 according to EU regulations.

Data base development

Since international experts recommended that a data base of around 200 chemicals is required for the assessment of test performance, it was agreed by the participants of phase I and by the sponsor BMFT, to conduct a two year database development study. In phase II 166 coded chemicals were tested in the two *in vitro* assays, each of them in two laboratories. Test chemicals backed by Draize eye test data obtained according to OECD guideline 405 were provided by industry and selected to represent a wide spectrum of chemical classes and eye irritation properties. At the end of phase II independent quality control of *in vitro* and *in vivo* data and biostatistical evaluation of the results turned out to require more resources than anticipated. Therefore, in a subsequent joint BMFT project on biostatistics in 1993 the quality of all of the *in vivo* and *in vitro* data of the present study was evaluated independently. In the quality assurance step, which is an essential prerequisite for biostatistics, the number of chemicals backed by high quality data was reduced to 143.

Tiered *in vitro/in vivo* testing strategy

In *vitro/in vivo* correlation for the classification of R-41 chemicals was insufficient with data from the NRU CT test. Classification of severely eye irritating chemicals according to the HET-CAM scoring system developed during phase I of the study was also insufficient. Discriminant analysis of 10 endpoints of the HET-CAM test and the NRU CT test revealed that the detection time of coagulation, the most severe reaction on the CAM, was significantly better suited to identify severely eye irritating properties of test chemicals than any other endpoint. The predictive power of this endpoint of the HET-CAM test was improved when combined with the endpoint IC-50 of the NRU CT test. For water soluble chemicals the combination of the NRU IC-50 and the HET-CAM mtc10, the detection time for coagulation after application of a 10% solution, had the highest discriminant power to identify R-41 chemicals. For less water soluble chemicals the HET-CAM mtc100, the detection time of coagulation after application of the undiluted chemical had the highest predictive power. Using these endpoints

of the HET-CAM and the NRU CT test, dicriminant analysis allowed to develop a tiered testing strategy to identify severely eye irritating chemicals which are labelled R-41, without testing on rabbits. Only chemicals, which are not identified in the HET-CAM and NRU CT test have to be tested in the Draize eye test in 1-3 rabbits. The tiered *in vitro/in vivo* testing strategy developed and validated in the present study is significantly contributing to a reduction of severely eye irritating materials in the Draize eye test. The tiered approach has been accepted by the competent authorities in Germany in 1994 to classify severely eye irritating chemicals and finished products.

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Hepatocyte bioreactors for *in vitro* studies on drug metabolism in the pharmaceutical industry as an alternative to animal testing

Abstract

The toxicological and pharmaceutical evaluation of new substances is essential in human risk assessment and drug development. So far, studies aimed at assessing the safety of a potential new medical product before its first administration to man are carried out in laboratory animals, except for certain genotoxicity tests, in compliance with European Directives. Reducing the utilisation of animals in the pharmaceutical industry would address ethical issues in our society. For this purpose, new in vitro *techniques are required which enable toxicity and metabolism studies on new drugs without jeopardising human safety.*

In a multidisciplinary approach, a new technology for in vitro *liver cell culture was developed combining highly specialised bioreactor technology with semi-automated, variable perfusion elements.*

Within the bioreactor, three independent capillary systems provide continuous gas supply, medium supply and removal of metabolic products. The capillaries are inter-woven to build a tight network, enabling decentralised oxygen and nutrition supply with low gradients. Pores within the capillary walls allow medium and gas transfer to the cells adhering to the outside of the capillaries. Different flow paths can be chosen according to the actual requirements of the cells.

The construction of the perfusion system is based on a modular principle employing independent units to enable individual modifications and easy upscaling of the system. Pressure-regulated pump control units and a temperature-controlled heating unit enable stable pressure and temperature conditions. Flow-meters for oxygen and carbon dioxide supply allow continuous monitoring and control of the gas flow and composition. The separation of medium supply and medium recirculation pumps facilitates the application of different experimental set-ups.

Various study objectives for predictive studies on drug toxicity and metabolism within the early pre-clinical phase of drug development can be addressed:

- *single administration studies, e.g. studies on "acute" toxicity, metabolite patterns and metabolism rates,*
- *repeated administration studies, e.g. studies on "chronic" toxicity and enzyme induction effects,*
- *continuous exposure studies, e.g. studies on "chronic" toxicity, carcinogenesis, enzyme induction, drug-drug-interactions and steady-state conditions,*
- *studies on first pass and clearence effects.*

To date the system has been applied successfully to the culture of primary human, pig and rat, and immortalised human hepatocytes (HepZ cell line). The long-term maintenance of specific phenotypic as well as functional properties of liver cells in the bioreactor has been demonstrated. Light and transmission electron microscopy studies have proven the three-dimensional re-organisation of hepatocytes and non-parenchymal cells of the liver to form tissue-like structures including extracellular matrix components produced by the cells. Formation of bile canaliculi and desmosomes by hepatocytes and the presence of sinusoid-like structures have been demonstrated. Furthermore, studies on primary human hepatocytes from organs not suitable for transplantation showed that even severely damaged cells can partly restore their capacity for protein synthesis and cytochrome P450-dependent xenobiotic metabolism under the conditions of bioreactor perfusion culture. Studies on immortalised human hepatocytes in the system, investigating proliferative activities and metabolic functions showed a high proliferation rate of the cells and, although at a low level, the expression of specific cytochrome P450 activities. Thus, the bioreactor model developed could be a potent tool for multiple applications in pre-clinical drug development and research. Moreover, the option for culturing human cells in the system allows the prediction of effects in man before its first clinical administration, which would lead to an increased safety of new drugs.

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