Grußadresse zum 13. MEGAT Kongress über „Alternativen zum Tierexperiment"

Sehr geehrte Kongressteilnehmer(innen),

im Namen von zet, der österreichischen nationalen Plattform für Alternativen zum Tierexperiment, darf ich Sie herzlich zum diesjährigen „Linzer Kongress" begrüßen.

Die Vorverlegung des Treffens von September auf Juni hat mit der EU-Ratspräsidentschaft Österreichs zu tun.

Anlässlich der letzten (ersten) Ratspräsidentschaft hat Österreich ein Symposium zur „Implementierung der 3R in der Europäischen Union in Wissenschaft und Industrie" veranstaltet. Diese Aktivität wird nun anlässlich der diesjährigen Ratspräsidentschaft mit dem „13. MEGAT/zet-Kongress über Alternativen zum Tierexperiment" fortgesetzt.

Das Organisationskomitee hat sich deshalb bemüht, das Programm so zu gestalten, dass ein möglichst großer Kreis von Anwendern alternativer Methoden in ganz Europa angesprochen wird und hat deshalb Englisch als Kongresssprache gewählt.

Die Tagungsteilnehmer werden über die Bemühungen der EU zur Implementierung alternativer Methoden in Grundlagenforschung, Industrie einschließlich der Kosmetik-Industrie, und auf Behördenebene, informiert. Dabei werden, wie bisher, ethische und rechtliche Aspekte von Tierversuchen ebenso wie die mit der Etablierung standardisierter *in vitro* Verfahren (Zellkulturtechniken) verbundenen Probleme behandelt.

Wichtig erschien es uns auch, die neuesten Entwicklungen bezüglich der Richtlinie für die neue Chemikalienpolitik der EU (REACH) im Zusammenhang mit dem Einsatz von Alternativen zu beleuchten.

Nicht zuletzt nimmt die Präsentation und Diskussion neuer Entwicklungen zur Implementierung der 3R in der biomedizinischen Grundlagenforschung und der angewandten Forschung breiten Raum ein.

Wie bisher wird bewusst auf Parallelsitzungen am Kongress verzichtet, dafür aber den Posterpräsentationen mehr Zeit gewidmet, sodass die präsentierten Ergebnisse intensiv diskutiert werden können. Die besten und innovativsten Poster werden mit einem Preis ausgezeichnet, um mehr junge Wissenschafter zur Weiterentwicklung der 3R Prinzipien zu motivieren.

Wir hoffen, Ihnen ein attraktives Programm in einer der innovativsten österreichischen Städte bieten zu können und freuen uns auf eine zahlreiche Teilnahme!

Ihr

Walter Pfaller, zet – Vorstand

Welcome address to the 13th MEGAT-Congress on "Alternatives to Animal Experimentation"

Dear Participant,

on behalf of zet (Centre for Alternative and Complementary Methods to Animal Testing), the Austrian national platform on Alternatives, I cordially welcome you to this year's "Linz-Congress". The unusual date, June instead of September, was chosen to lie within the Austrian EU-Presidency.

On occasion of the previous, first Austrian EU-presidency, a symposium on "Implementation of the 3R Targets in the EU, in Science and Industry" was held by the Austrian authorities. This activity will be continued during the current EU-presidency of our country in form of the "13th Congress on Alternatives to Animal Experimentation".

The organising committee has invested all its efforts in order to address the largest possible number of scientists from all over Europe that are potentially interested in this field and has thus, for the first time, selected English as the official congress language.

The meeting participants will be updated on the European Community's and the member states' efforts to implement the 3R principles into academic, i.e. basic biomedical, and applied, i.e. industrial (including the cosmetics industry), research and on the status of implementation by European Regulatory Authorities. In this context, the programme will focus on legal and ethical aspects as well as on problems arising from efforts to standardise and harmonise *in vitro* approaches, specifically cell culture techniques.

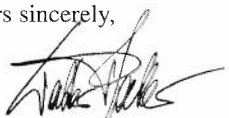
We further thought it of importance to review the newest developments in the guidelines on European chemicals and cosmetics with regard to the use of alternatives.

Last but not least the programme will pay special attention to new technologies and methods promising to expand our repertoire of 3R related experimental approaches.

As in the past, we will consciously avoid parallel sessions at the congress, but instead give a higher value than in the past to poster presentations. The best and most innovative posters will receive awards, which should help to convince and motivate young scientists to contribute effectively to the development of alternative methods in the future.

We strongly hope to be able to provide you with an attractive programme in one of the most innovative cities of Austria. We are looking forward to welcoming a large number of participants and scientists interested in promoting 3R related research.

Yours sincerely,



Walter Pfaller, Head of zet

Congress Linz 2006

Preliminary program

Friday, 02.06.2006

12.45-13.00 Addresses of welcome

Session I **REACH, cosmetics and toxicology**
Chair: Pfaller Walter, A-Innsbruck
N.N.

13.00-13.20 Sauer Ursula, D-Neubiberg
Finalizing REACH – An evaluation of the European Parliament's and the Council's position from the point of view of the German Animal Welfare Federation

13.20-13.40 Hartung Thomas, I-Ispra
Finalizing REACH – An evaluation of the European Parliament's and the Council's position from the point of view of European Commission/ECVAM

13.40-14.00 Lucaroni Beatrice, B-Brussels
N.N.

14.00-14.15 Hendriksen Coenraad, NL-Bilthoven
Towards eliminating the use of animals for regulatory required vaccine quality control

14.15-14.30 Mueller Stefan O., D-Darmstadt
Alternatives in pharmaceutical toxicology: global and focussed approaches

14.30-14.45 Westmoreland Carl, UK-Sharnbrook
N.N. (Amendment to the Cosmetics Directive)

14.45-15.00 Ruhdel Irmela, D-Neubiberg
N.N. (Amendment to the Cosmetics Directive)

15.00-15.40 Coffee-break and posters

Session II **Ecotoxicology**
Chair: Cervinka Miroslav, CS-Hradec Kralove
Scheiwiller Susanne, CH-Zurich

15.40-16.00 Hoet Peter, B-Leuven
Nanoparticles – a new challenge to toxicology

16.00-16.15 Kahru Anne, EST-Tallinn
Ecotoxicological tests and recombinant luminescent microbial models in toxicity studies: contribution to 3Rs

16.15-16.30 Tonkopii Valerii, RUS-St. Petersburg
Daphnia magna as alternative bioobject in ecotoxicology

Session III **Education**
Chair: Cervinka Miroslav, CS-Hradec Kralove
Scheiwiller Susanne, CH-Zurich

16.30-16.50 Dewhurst David, UK-Edinburgh
Computer-based alternatives – past, present and future

16.50-17.05 Boumans Iris, NL-Utrecht
Humane endpoints in laboratory animal experimentation: an interactive CD ROM

17.05-17.20 Martinsen Siri, S-Glava
Veterinary training without the use of laboratory animals – an example from Norway

17.20-17.35 Jukes Nick, UK-Leicester
Internationalising alternatives in higher education

17.35-19.30 Postersession I

20.00 Reception (at the congress venue)

Saturday, 03.06.2006

Session IV **Chronic toxicity**
Chair: Hartung Thomas, I-Ispra
N.N.

09.00-09.15 Runge Dieter, D-Schwerin
Use of a standardized and validated long-term human hepatocyte culture system for repetitive analyses of drugs: Repeated administrations of acetaminophen reduces albumin and urea secretion

09.15-09.30 Thedinga Elke, D-Rostock
Using of an *in vitro* system for prediction of hepatotoxic effects in primary human hepatoctyes

09.30-09.45 Cervinka Miroslav, CS-Hradec Kralove
Microscope-assisted cytometry as an *in vitro* alternative for sub-chronic toxicity assessment

09.45-10.00 Slaughter Mark, UK-Sandwich
10-day medium throughput *in vitro* screen to assess chronic cytotoxicity of nucleoside analogues

10.00-10.15 Giese Christoph, D-Berlin
Human organoids for immunogenicity and immunotoxicity testing

10.15-10.30 Huang Song, CH-Geneva
A novel *in vitro* cell model of muco-ciliated human airway epithelium for long term toxicity testing

10.30-10.45 Poth Albrecht, D-Rossdorf
The Bhas42 cell transformation assay as an predictor of carcinogenicity

10.45-11.15 Coffee-break and posters

Session V **Acute and target organ toxicity**
Chair: Spielmann Horst, D-Berlin
N.N.

11.15-11.30 Telang Nitin, USA-New York
Cell culture model for colon carcinogenesis, cemoprevention and organ-selective-toxicity

11.30-11.45 Szameit Sandra, A-Seibersdorf
Application of DNA microarrays for immunotoxicological testing

11.45-12.00 Scholz Gabriele, CH-Lausanne
Hazard identification in chemical food safety: use of modeling, primary hepatocyte cultures and microarrays to address the level of concern for potential process contaminants of unknown toxicity

12.00-12.15 Hoffmann Jens J., D-St. Katharinen
Increased reproducibility in toxicity testing using Epidermal Skin Test 1000

12.15-12.30 Haltner-Ukomadu Eleonore, D-Saarbrücken
Assessment of applicability and tolerability of drugs and excipients on lung cell models

12.30-14.00 Lunch break

Session VI **Ethical and legal aspects**
Chair: Roman Kolar, D-Neubiberg
Mayr Petra, A-Linz

14.00-14.20 Van der Valk Jan, NL-Utrecht
Criteria for expert assessment by animal experiments committees

14.20-14.40 Kuil Janne, NL-The Hague
Regulatory animal testing

14.40-15.00 Ferrari Arianna, D-Tübingen
Schwierigkeiten und Dringlichkeit der Evaluierung der gentechnischen Veränderung von Tieren nach den 3R-Prinzipien

15.00-15.20 Lindl Toni, D-Munich
Animal experiments in biomedical research. An evaluation of the clinical relevance of approved animal experimental projects: No evident implementation in human medicine within more than 10 years

15.20-15.40 Luy Joerg, D-Berlin
Ein Lebensrecht für Tiere – ethisch zu rechtfertigen?

15.40-16.00 Balluch Martin, A-Vienna
Tiere haben ein Lebensrecht

16.00-18.00 Postersession II

18.15 **MEGAT – general assembly**

20.00 **Social evening & awards ceremony**

Sunday, 04.06.2006

Session VII **Good Cell Culture Practice**
Chair: Gstraunthaler Gerhard, A-Innsbruck
Fischer René, CH-Zurich

09.00-09.10 Gstraunthaler Gerhard, A-Innsbruck
Safety in the cell culture laboratory

09.10-09.30 Merten Otto-Wilhelm, F-Evry
Contaminations caused by viruses and prions

09.30-09.50 Weiss Richard, A-Salzburg
Viral expression vectors: biosafety considerations

09.50-10.10 Fischer René, CH-Zurich
A new high potent freezing medium closes the last gap in the "serum free cell culture technique"

Session VIII **Free communications**
Chair: Gstraunthaler Gerhard, A-Innsbruck
Fischer René, CH-Zurich

10.10-10.25 Vuia Alexander, D-Berlin
Results of the validation study on percutaneous absorption via reconstructed human epidermis

10.25-10.40 Visan Anke, D-Berlin
Estimation of the embryotoxic potency of valproic acid derivatives *in vitro* by using the Embryonic Stem Cell Test (EST)

10.40-10.55 Eder Claudia, A-Vienna
A modified HET-CAM approach for biocompatibility testing of medical devices

10.55-11.10 Falkner Erwin, A-Vienna
HET-CAM model for melanom tumorgenicity studies

11.10-11.40 Coffee-break and posters

Session IX **New methods**
Chair: Haltner-Ukomadu Eleonore, D-Saarbrücken
N.N.

11.40-12.00 Schrattenholz André, D-Mainz
Stem cell- based *in vitro* models as a basis to test efficacies of preclinical phases of anti-neurodegenerative treatments

12.00-12.20 Curren Roger D., USA-Gaithersburg
A novel, non-animal genetic toxicology assay – the reconstructed skin micronucleus assay using EpiDerm™

12.20-12.40 Humphery-Smith Ian, UK-Newcastle
Knowledge of the Human genome (total DNA sequence) as an opportunity to build better drugs and concomitantly reduce the need for animal experimentation

12.40-13.00 Pfaller Walter, A-Innsbruck
EpiFlow perfusion culture improves *in vitro* assessment of acute and chronic toxicity

13.00-13.20 Vedani Angelo, CH-Basel
The challenge of predicting drug toxicity *in silico*

13.20-13.35 Weitzer Georg, A-Wien
An *in vitro* model for early embryonic development

13.35-13.50 Hayess Katrin, D-Berlin
Expansion of the Embryonic Stem Cell Test: Differentiation into neural cells

13.50 **Closing words**

Poster

Use of an *in vitro* skin model to study the dermo-epidermal junction

Núria Almiñana and Eva Martinez

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E-mail: nalminana@lipotec.com

Some years ago, prior to human use of new cosmetic products, *in vivo* animal tests were conducted in order to screen for safety and efficacy. The tenets of ethical animal testing call for practice of the three R\'s of refinement, reduction and replacement of animal tests. Therefore, a growing need exists for rapid and reliable *in vitro* methods for safety and efficacy screening of new dermato-cosmetological products. Three-dimensional organotypic skin models exhibit *in vivo*-like morphological, metabolical, and growth characteristics which are uniform and highly reproducible. These skin-like tissues consists of organized basal, spinous, granular and cornified epidermal layers analogous to those found *in vivo*, and they can provide alternative models to replace animal and human experiments. In the present study, these organotypic cultures have allowed to determine the effects of the test product in the basement membrane of skin, also called dermo-epidermal junction, which is responsible of the contact between epidermis and dermis, and plays an important role in the maintenance of tissue architecture and normal skin functions. In the epidermis of the skin, basal keratinocytes adhere to the basement membrane through α6β4 integrin which binds to Laminin-5, an interaction necessary for hemidesmosome formation and basement membrane stability. Hemidesmosomes are anchoring junctions of the cell to a non-cellular substrate that play a critical role in stabilising the association of the dermis with the epidermis. The importance of the hemidesmosome junctions for the epidermis attachment is clearly demonstrated by the fact that in a variety of blistering diseases, there is a disruption of the hemidesmosomal complex. Several studies also confirm that there are ch anges occurring at this dermal-epidermal junction during the course of age. Serilesine is a Laminin-like peptide that was found to be active in promoting fibroblast and keratinocyte proliferation and therefore this peptide may play an important role in skin. In this study, Serilesine has been tested in order to determine its effects on the basement membrane proteins and on hemidesmosome formation. To this purpose, the effect of Serilesine on Laminin-5 and α6-integrin expression and hemidesmosome formation was determined in the skin model.

Keywords: skin models, dermo-epidermal junction, laminin, integrin

Poster

The human hepatoma HepaRG cells : A new *in vitro* model for xenobiotic metabolism and toxicity studies in human liver

Caroline Aninat, Denise Glaise, Marc Levee, Christophe Chesne, Fabrice Morel. Olivier Fardel, Christiane Guguen-Guillouzo and André Guillouzo

INSERM U620 and INSERM U522, University of Rennes, France
E-mail: marion.gibert@biopredic.com

Biopredic International Rennes, France Human primary hepatocytes and hepatoma cells are widely used for *in vitro* pharmaco-toxicological studies. Although primary hepatocytes represent the most pertinent system, they have limitations due to scarce and unpredictable availability, early phenotypic changes and low growth activity and life-span. Hepatoma cell lines have indefinite proliferative growth but they usually lack a variety of functions, especially the major CYPs involved in xenobiotic metabolism, the constitutive androstane receptor (CAR) and several drug transporters. We have analysed the drug metabolic capacity of the HepaRG cell line derived from a human hepatocellular carcinoma (1). When HepaRG cells are plated at low density, they actively divided and after having reached confluence, form typical hepatocyte-like clusters surrounded by biliary epithelial-like cells. Transcripts encoding a number of genes related to drug metabolism were estimated by RT-qPCR. All were detected in confluent HepaRG cells and for most of them, at levels comparable to those measured in primary human hepatocyte cultures; they included various CYPs (1A2, 2B6, 2C9, 2D6, 2E1 and 3A4), phase II enzymes (UGT1A1, GSTA1, A4 and M1), nuclear receptors (AhR,) and sinusoidal (OCT1, OATP-C, NTCP and BSEP) and canalicularaPXR, CAR and PPAR (MDR1, MRP2 and MRP3) drug transporters. The levels of several transcripts were strongly increased when HepaRG cells were maintained for 2 weeks at confluence in the presence of 2% DMSO. By contrast, in confluent HepG2 cells tra nscripts of CYP1A2, CYP2B6, CYP2E1, CYP3A4, CAR, OCT1, OATP-C, NTCP and BSEP were barely expressed in any (2,3). Basal CYP activities and response to prototypical inducers as well as activities of drug transporters and regulation by known inducers confirmed the functional resemblance of HepaRG cells to primary human hepatocytes. These results show for the first time that the major CYPs, CAR and hepatic drug transporters can remain expressed in a human hepatoma cell line, leading to the conclusion that HepaRG cells represent a unique tool for the study of human liver metabolism and toxicity of chemicals.

1) Gripon, P. et al. 2002. Infection of a human hepatoma cell line by hepatitis B virus. *Proc. Natl. Acad. Sci. U S A 99*, 15655-15660. 2) Aninat, C. et al. 2006. Expression of cytochromes P-450, conjugating enzymes and nuclear receptors in human hepatoma HepaRG cells. *Drug. Metab. Dispos. 34*, 75-83. 3) Le Vee, M. et al. Functional expression of sinusoidal and canalicular hepatic drug transporters in the differentiated human hepatoma HepaRG cell line; *Europ. J. Pharmaceut. Sci*. in press.

Keywords: hepatic cell line, metabolism and toxicity studies

Poster

Non-human primates in medical research and drug development: A critical review

Jarrod Bailey

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There is much current debate surrounding the use of non-human primates (NHPs) in medical research and drug development, largely from an ethical perspective. Despite a significant proportion of the European public objecting to NHP experiments regardless of any potential human benefits, the general consensus seems to be supportive providing that it results in tangible medical progress and if there is no alternative. This review, stimulated by calls for evidence from UK-based inquiries into NHP research, takes a critical view in order to provide some important balance against papers supporting it and calling for it to be expanded. We show that there is a paucity of evidence to demonstrate the positive contribution and translation of NHP research to human medicine, that there is a great deal of often over-

looked data showing NHP research to be irrelevant, unnecessary, even hazardous to human health and to have little or no predictive value and application to human medicine. We briefly discuss the reasons why this may be so, reflect upon the consequences for future medical progress, and on the basis of our findings suggest a more scientifically robust and promising way forward.

Keywords: Primates, NHP, monkeys

Poster

Laboratory routines cause animal stress

[NB: recently published in Contemporary Topics in Laboratory Animal Science Nov. 2004;43(6):42-51]

Jonathan Balcombe, Neal Barnard and Chad Sandusky.

Physicians Committee for Responsible Medicine, Washington DC, US.
[to be presented at Linz by Andrew Knight from Animal Consultants International if no co-authors can attend]
E-mail: jbalcombe@pcrm.org

Eighty published studies were reviewed to document the potential stress associated with three routine laboratory procedures commonly performed on animals: handling, blood collection, and gavage. Handling was defined as any non-invasive manipulation that is part of routine husbandry, such as picking up an animal, and/or cleaning or moving an animal's cage. Significant changes in stress indicators (e.g., concentrations of corticosterone, glucose, growth hormone or prolactin, heart rate, blood pressure, and/or behavior) were associated with all three procedures in the reviewed studies (reporting primarily on rats, mice, monkeys, dogs, rabbits, hamsters, bats, or birds). Studies showed that animals responded with rapid, pronounced, and statistically significant elevations in stress-related responses to each of the procedures examined. Changes from baseline or control measures typically ranged from 20 to 100 percent or more and lasted from 30 to 60 min or more. These findings indicate that laboratory routines are associated with stress, and that animals do not readily habituate to them. The data suggest that significant fear, stress, and possibly distress are predictable consequences of routine laboratory procedures, and that these phenomena have substantial scientific and humane implications for the use of animals in laboratory research.

Keywords: Animal experiment, stress, fear, handling, blood collection, gavage

Poster

Laboratory environments and rodents' behavioural needs: A review

[NB: to be published in Laboratory Animals 2006. In press.]

Jonathan Balcombe

Physicians Committee for Responsible Medicine, Washington DC, US. [to be presented at Linz by Andrew Knight from Animal Consultants International if Dr Balcombe is unable to attend]
E-mail: info@animalconsultants.org

Laboratory housing conditions have significant physiological and psychological effects on rodents, raising both scientific and humane concerns. Published studies of rats, mice and other rodents were reviewed to document behavioural and psychological problems attributable to predominant laboratory housing conditions. Studies indicate that rats and mice value opportunities to take cover, build nests, explore, gain social contact, and exercise some control over their social milieu, and that the inability to satisfy these needs is physically and psychologically detrimental, leading to impaired brain development and behavioural anomalies (e.g., stereotypies). To the extent that space is a means to gain access to such resources, spatial confinement likely exacerbates these deficits. Adding environmental "enrichments" to small cages reduces but does not eliminate these problems, and we argue that substantial changes in housing and husbandry conditions would be needed to further reduce them.

Keywords: animal experiment, laboratory animal housing, stress, environmental enrichment

Lecture

[Animals have a right to live]
Tiere haben ein Lebensrecht

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Staat und Kirche sind zu trennen, und daher dürfen sich auch die Gesetzgebung und das Rechtssystem nicht an religiösen Dogmen orientieren. Sie müssen auf einer empirisch-rationalen Ethik basieren, die konsensfähig ist. Dazu bedarf es zunächst zweierlei. Erstens muss das Bewusstsein naturwissenschaftlich gefasst und beschrieben werden. Und zweitens muss der Anthropozentrismus und das darauf basierende Mensch-Tier Bild der Aufklärung kritisiert und auf seine faktische Basis hin untersucht werden. Dabei zeigt sich, dass Bewusstsein an sich durch die Fähigkeit zu Autonomie bestimmt ist. Und Bewusstsein hat eine evolutionäre Kontinuität, d.h. es gibt keinen grundsätzlichen Unterschied zwischen menschlichem und tierlichem Bewusstsein. Eine implizite Voraussetzung dafür, autonom handeln zu können, ist, am Leben zu bleiben. Das Leben ist von höchstem instrumentellem Wert für ein autonomes Lebewesen, um seine Autonomie umsetzen zu können, und liegt daher in seinem größten Interesse. Umgekehrt ist daher der Tod von größtem Schaden für dieses Lebewesen. Weil ich als autonomes Lebewesen autonom handeln will, fordere ich u.a. von der Gesellschaft ein Recht auf Leben ein. Wenn ich rational konsistent bin, dann muss ich dasselbe Recht auch für alle anderen autonomen Lebewesen, die autonom handeln wollen, fordern. Mein Recht auf Leben – ein Menschenrecht – wiegt also genauso viel, wie das Recht auf Leben für alle anderen Wesen mit Bewusstsein. Welche Wesen außer mir noch ein Bewusstsein haben, bleibt als rein empirische Frage zu klären, aber zumindest alle Wirbeltiere und Kopffüßer dürften darunter fallen.

Keywords: Tierrechte, Bewusstsein, Mensch-Tier Beziehung

Poster

A metabolic activation system for the embryonic stem cell test

Markus Binder, Jutta Volland, Frauke Meyer, Ulrich Hübel and Paul-Georg Germann
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The Embryonic Stem Cell Test (EST) determines the embryotoxic potential of chemicals. The aim of this study is to add a static co-culture system with metabolic competence to this test to be able to detect pro-teratogens. The EST is based on the capacity of embryonic stem cells to differentiate *in vitro* into contracting cardiomyocytes which can be detected by microscopic analysis. It is the only *in vitro* reproductive toxicity test without using test animals and has been scientifically validated by the European Centre for the Validation of Alternative Methods (ECVAM). In order to be accepted by international authorities, the EST must be able to detect pro-teratogens which need metabolic activation to exert a toxic action.

Different metabolic systems were tested: primary hepatocytes from rat and mouse, and the hepatoma cell lines H4IIE (rat), Hepa 1-6 (mouse) and HepG2 (human). They were selected considering their tolerance of the test environment, and a high metabolic competence. Investigations included the time intervals in which metabolic activation has to take place, and different co-culture techniques at different test phases. Cyclophosphamide, a known pro-teratogen, was used as a first model substance.

We found that metabolic activation must take place for the whole test period. Changing the test duration or the point of treatment reduces the test sensitivity as tested in the EST with 5-fluorouracil and 6-aminonicotinamide. In hepatocyte medium, embryonic stem cells do not differentiate into beating cardiomyocytes, and primary hepatocytes do not tolerate embryonic stem cell medium. Hepa 1-6 cells show abnormal morphology on co-culture membranes. Hence, H4IIE- or HepG2 cells are the most promising candidates. With these cells, the maintenance of a co-culture in the EST is possible. First results suggest a metabolic activation of cyclophosphamide.

It could be shown that in the EST, a co-cultivation with hepatoma cells is possible. The test protocol has yet to be optimised to reliably meet all validity criteria. In further studies, valpromide and retinol will be tested in the metabolic competent EST (mEST) to test its proper function.

Keywords: embryonic stem cell test, metabolic activation, hepatocytes, co-culture

Lecture

Humane endpoints in laboratory animal experimentation: an interactive CD ROM

Iris J. M. M. Boumans and Coenraad F. M. Hendriksen
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Humane endpoints are an example of a refinement alternative (one of the 3Rs: Replacement, Reduction and Refinement). The aim of a humane endpoint is to terminate, minimise or reduce an animal's pain and/or distress. The CD ROM "Humane endpoints in laboratory animal experimentation" is an interactive program for educational and training purposes and is intended to be part of the trainings program of animal welfare officers, researchers, animal technicians and animal caretakers. The CD ROM focuses on more aspects than only the title does expect. The first part of the CD ROM contains chapters on normal behavior of a mouse or rat, pain and distress and recognition of general clinical signs as well as clinical signs typical for a number of specific biomedical research areas (e.g. cancer research). Another part of the CD ROM offers information about pathology, which can be a useful control tool for the correct assessment of suffering and the quality of the experiment itself. This part is followed by the main topic of the CD ROM: Humane endpoints, which describes the notion "humane endpoints" and gives an overview of parameters for applying humane endpoints. The responsibilities and validation of humane endpoints are discussed, and relevant national and international laws, guidelines and reports are also included. The last part gives the opportunity to test the acquired knowledge by a number of interactive tests about different subjects. Furthermore the CD ROM includes a glossary and more than one hundred additional images and video clips are available. The Dutch version of the CD ROM has been distributed in the Netherlands since 2004 and is nowadays being used by the research community and also in a few courses (e.g. Laboratory Animal Science Course). The English version is finished in the beginning of 2006 and will be available at the 13th Congress on Alternatives to Animal Testing in Linz.

Keywords: Humane endpoints, refinement, education, rodents

Poster

The animal use barometer

Iris Boumans[1, 2] and Coenraad Hendriksen[2]
[1]Sophia Vereeniging, Amsterdam and [2]Netherlands Centre Alternatives to Animal Use, Utrecht University, The Netherlands
E-mail: I.Boumans@vet.uu.nl

Annual figures on the use of laboratory animals in biomedical research are the net result of all factors that determine animal studies and that increase or decrease total numbers. Based on financial support of the Sophia Vereeniging; a liberal animal welfare organisation in the Netherlands, we evaluated trends in animal use over the years, particularly for the Netherlands, but also for the UK, Switzerland and the EU. Parameters included total animal numbers, species used, purposes, degree of pain and suffering, etc. We also looked at factors that influence the use of animals and current developments in these factors. We identified four main factors: society in general and NGO organisations in particular, industry, regulatory bodies and governmental/political organisations. Generally, influences are complex and quite often also conflicting. For example, society as a whole favours the EU programme REACH but is quite indifferent with regard to the number of chemicals to be tested. However, individual NGO's lobby to increase the number (such as Environmental organisations), while animal welfare organisations want just the opposite. As the number of animals is related to the number of chemicals tested, animal usage will be influenced by those activities. Specific attention is given to the Three R's. The route of a Three R method from the stage of development to validation and final implementation is being analysed and potential obstacles are identified. The report includes many recommendations for a more efficient Three R's strategy. We believe that a better understanding of all those factors that influence laboratory animals use, as well as knowledge of the processes that underlie Three R's output will be beneficial for a more specific and effective policy towards smaller numbers of animals and higher welfare standards for those animals still to be used.

Keywords: three Rs, development animal use

Poster

[Construction of a three-dimensional tubular trachea-model] Aufbau eines dreidimensionalen tubulären Trachea-Modells

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Zellsysteme gewinnen als Alternative zum Tierversuch immer mehr an Bedeutung. Solche 3D-Modelle wurden bereits für die Cornea und die Haut etabliert und eignen sich aufgrund ihrer organspezifischen Eigenschaften zur Testung verschiedenster Substanzen hinsichtlich ihrer Biokompatibilität. Auch die Atemwege stellen eine wichtige Eintrittspforte für verschiedenste Krankheitserreger und Umwelteinflüsse dar. Zielgerichtet wollen wir ein tubuläres Testsystem entwickeln, in dem die Zellen unter physiologischen Bedingungen in einem Bioreaktor kultiviert werden. Zum Aufbau eines 3D-Modells der *Trachea* werden zunächst porcine Zellen mit unterschiedlichen enzymatischen Methoden isoliert und eine Kollagen I-Trägerstruktur beschichtet. Die Besiedelung erfolgt anschließend bei 37° C und 5% CO_2. Zur Versorgung der Zellen wird Airway Epithelial Cell Growth Medium mit verschiedenen Supplementen eingesetzt. Die Zellcharakterisierung wird über histologische und immunhistologische Methoden und die Vitalität über Live Dead Essays festgestellt. Zur Isolierung der Trachea-Zellen eignet sich unter anderem die Auswachsmethode, eine weitere Möglichkeit stellt die enzymatische Isolierung der Zellen dar. Dabei wurden die verschiedenen enzymatischen Methoden unter Verwendung von Trypsin, Collagenase und Protease verglichen. Bereits 24 h nach der Isolation lässt sich die charakteristische Kinozilienbewegung erkennen. Unser Ziel ist der Aufbau eines komplexen 3D-Modells einer bioartifiziellen Trachea in tubulärer Form, durch die im Bioreaktor unter zellphysiologischen Bedingungen die Atmung simuliert werden kann. Dieses Modell könnte dann als Ersatz zu Tierversuchen oder auch als biologisches Implantat, welches die Gewebefunktion erhält, eingesetzt werden. Erste Arbeiten zur Langzeitkultivierung von porcinen Trachea-Zellen *ex-vivo* liegen bereits vor, eine Umsetzung des Modells mit humanen Zellen wird angestrebt.

Keywords: Trachea, Dreidimensionales Testsystem, primäre Zellen

Lecture

Microscope-assisted cytometry as an *in vitro* alternative for sub-chronic toxicity assessment

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Sub-chronic and chronic toxicity of xenobiotics is standartly assessed using laboratory animals and, so far, no alternative methods have been generally accepted to replace this testing strategy. This area of research is therefore were challenging, in particular in view of the recent developments in the field of alternatives. Of all proposed and evaluated alternative models, the development of a suitable *in vitro* system for long term (5 and more days) toxicity assessment of xenobiotics by monitoring various cellular functions represents an attractive challenge. In order to develop and successfully validate and implement such an *in vitro* system, several questions, which may be divided roughly into two groups, are to be solved. Firstly, it is the biological nature of any *in vitro* system which has to be considered, in particular its growth properties, need for passaging and maintenance of integrity. Secondly, technical aspects of thus proposed model require the use of a very sophisticated monitoring system capable of non-invasive observation of cellular behavior and various cellular functions. In ideal case, long term monitoring of selected cellular functions and subsequent data mining should combine various optical methods (light microscopy – phase contrast, differential interference contrast) following basic parameters such as cell morphology, cell proliferation, motility or cell death. Furthermore, this system should use fluorescence to determine other aspects of cellular functions (oxygen consumption, mitochondrial activity, gene e xpression and so forth) and their potential perturbation after exposure to studied xenobiotics. In our presentation, we will

present and discuss advantages and drawbacks of a combination of several approaches – classical phase contrast time-lapse recording and advanced cytometry observation with Live Cell Imaging System (CellR, Olympus) and laser scanning confocal microscope with spectral analysis (C1si, Nikon). We will also focus on other associated technical problems including the use of optimal cultivation chambers, maintenance of standard experimental conditions and robustness of this approach towards sub-chronic and chronic *in vitro* toxicity experimentation.

This work was supported by Ministry of Education Research Project MSM 0021620820.

Keywords: sub-chronic toxicity, in vitro alternative, cytometry, time-lapse, microscopy

Lecture

A novel, non-animal genetic toxicology assay – the 3-D reconstructed skin micronucleus assay using EpiDerm™

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The 7th amendment to the Cosmetics Directive precludes the use of *in vivo* assays for genotoxicity assessments of cosmetic ingredients. This (among other animal testing prohibitions) poses a problem for the complete safety assessment of such materials. For most cosmetics, skin has the highest exposure; therefore we are developing an *in vitro* human skin micronucleus assay using the 3-D EpiDerm™ skin model (MatTek Corp, Ashland, MA). Theoretically such a model could approximate the complexities typical of *in vivo* exposures, e.g. absorption, tissue specificity, metabolism, etc., and at the same time reflect human-specific responses in these parameters. Our assay is based on applying two 10 ul doses of test material to the surface of the EpiDerm™ tissue 24 hours apart, and harvesting 24 hours after the last dose. Using this procedure we show dose related increases in both cytotoxicity and micronuclei induction for several model genotoxins including mitomycin C (maximum micronucleus response [MMR] ~8% at 0.6 ug total dose), vinblastine sulfate (MMR ~4% at 0.01 ug total dose), methylmethane sulfonate (MMR ~0.6% at 20 ug total dose), and N-methyl-N'-nitro-N-nitrosoguanidine (MMR ~0.7% at 40 ug total dose). The average background frequency of micronuclei is low at <0.2% (N=25). Three chemicals known to be non-skin carcinogens in rodents (4-nitrophenol, 1,2-epoxydedocane and trichloroethylene) do not induce micronuclei in our assay even when tested at cytotoxic doses

As the first step in investigating whether our model will respond to genotoxins requiring metabolic activation, we have shown that EpiDerm™ cultures from different donors express numerous genes associated with xenobiotic metabolism that are also found in normal human skin. We have also shown the inducibility of several of these genes by 3-methylcholanthrene. We believe this novel assay system holds excellent promise for future use as a human "*in vivo*-like" genotoxicity model for the assessment of both cosmetic and non-cosmetic materials.

Keywords: micronucleus, skin, in vitro, genetox, non-animal testing

Poster

The human hepatoma HepaRG cells: A promising tool for genotoxic studies of environmental chemicals

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Induced liver preparations known as S9 fractions are required for *in vitro* genotoxicity studies to activate indirect genotoxins. The use of S9 preparations, with mammalian cell cultures, raise toxicity problems. Moreover bias may arise due to the kinetic profiles (intra-cellular penetration) of short half-lives metabolites, produced by the extra-cellular S9 enzymes. In order to circumvent these limits, several genetically engineered immortal cells lines (ex: V79) have been developed with a stably expression of several functionally mammalian cytochromes P-450. We propose another approach, based on the use of a well equipped hepatic cell line. We have analysed the drug metabolic capacity of the HepaRG cell line derived from a human hepatocellular carcinoma, to establish its future potential in genotoxicity studies. When HepaRG cells are plated at low density, they actively divide and after having reached confluence, form typical hepatocyte-like clusters surrounded by biliary epithelial-like cells. Transcripts encoding a number of genes related to drug metabolism were estimated by RT-qPCR. All were detected in confluent HepaRG cells and for most of them, at levels comparable to those measured in primary human hepatocyte cultures; they included various CYPs (1A2, 2B6, 2C9, 2D6, 2E1 and 3A4), phase II enzymes (UGT1A1, GSTA1, A4 and M1), nuclear receptors (AhR,) and sinusoidal (OCT1, OATP-C, NTCP and BSEP) and αPXR, CAR and PPAR canalicular (MDR1, MRP2 and MRP3) drug transporters. Basal CYP activities and response to prototypical inducers as well as activities of drug transporters and regulation by known inducers confirmed the functional resemblance of HepaRG cells to primary human hepatocytes. These results show that the major CYPs, CAR and hepatic drug transporters can remain expressed in this human hepatoma cell line. HepaRG cells represent a promising tool for genotoxicity studies of environmental chemicals, more especially when the mutagenic potential of a chemical is related to its metabolites, or when the cellular enzymatic pathways, involved in the production of the genotoxins, are under evaluation.

Keywords: HepaRG, metabolism, genotoxicity, in vitro

Poster

Didactic assessments of educational models used as alternatives to harmful animal use

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When explaining to faculty that alternatives to animal use in education are preferable not only from an ethical point of view but also from an educational point of view, we need systematic assessments of the alternative models. This is consistent with evidence-based practice. Teachers are mostly interested in whether resources meet their learning objectives, but also in whether resources are interactive, realistic and user-friendly. Evaluations such as exams or assignments conducted to assess student achievement of learning objectives need to take into consideration the teaching methodology used, the contents, and the grouping of students. These didactic components are also included in an independent review process previously developed by the author at the European Resource Centre for Alternatives to animal use (EURCA). The review process is designed to enable teachers to systematically assess the educational value of alternative models. Other features examined include the co mparison between the alternative model and the animal laboratory, ease of use of the alternative model, the contribution of the resource to awareness of the 3Rs, and the level of service provided by the supplier. Several reviews of, mostly electronic, alternative models are used as examples. Generally, the reviewers are positive about the effectiveness of alternative models, but there is room for improvement both at didactic level, as well as the user level (navigation, presentation and level of interaction). Through teachers' assessments of educational models, alternative resources are becoming more accepted in mainstream education, which is likely to positively influence the attitudes towards the 3Rs of scientists, professionals and a new generation of science teachers.

Keywords: Alternative model, assessment, education, review

Lecture

Computer-based alternatives – past, present and future

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The use of computer-based alternatives to using animals in the teaching of disciplines such as pharmacology and physiology dates back to the early 1980's. Then there were two main approaches to development. Some programs were designed to simulate an animal preparation using a mathematical algorithm which predicted, based on known data, how a tissue would respond to drugs or drug combinations, and factors such as electrical stimuli. This type of program encouraged learning by exploration and was best suited to a learning environment in which a tutor was present to guide students. Another approach was to create a tutorial program around a set of data derived from a set of real experiments which had been designed by a knowledgeable tutor well acquainted with how a particular animal preparation could be used to teach major principles and factual knowledge. This second approach resulted in programs which could be used independent of tutor supoport though often learning would be enhanced by the presence of a tutor freed from troubleshooting technical problems with equipment. Each approach fosters different learning outcomes and both have been demonstrated to be extremely successful in differerent learning or teaching situations. Several studies have been carried out to evaluate the effectiveness of both types of program and evidence from these will be presented together with a description of successful strategies designed to enable integration of these resources into mainstream teaching.

Many of these computer programs are now quite old and, although the content and underlying pedagogical approach is still sound, the technology underpinning the delivery of the programs is rapidly becoming obsolete. A project to preserve the educational value of these programs in the face of rapidly changing delivery technologies is underway in Edinburgh. This aims to more effectively manage the component learning and information assets (text, images, animations, self-assessments, video and audio), provide teachers with easy-to-use authoring templates to enable them to build their own resources from these assets, and build future-proofed delivery engines compatible with delivery over the Internet or on CDROM. Examples of specific e-alternatives will be used to illustrate this process.

Keywords: computer-assisted learning (CAL), alternatives in teaching, computer simulations, animal experiments in teaching

Poster

Simulation of *in vivo* cell transplantation experiments using the HET-CAM bioassay

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Introduction: The HET-CAM (Hen Egg Test - Chorionallantoic Membrane) test system offers a partially immunodeficient, borderline *in vitro/in vivo* test system allowing the simulation of transplantation experiments prior to actual animal testing. Material and Methods: A collagen scaffold was seeded with sheep primary meniscus cells at different concentrations. Preliminary incubation *in vitro* was varied between three and fourteen days. Transplantation success and cell migration *in ovo* were evaluated using the HET-CAM assay. Results: Increasing cell seeding density from 2x105 to up to 5x106 cells per scaffold did not result in satisfying tissue formation at the transplantation site. Instead, the high cell density led to changed material properties of the scaffold, leading to poorer tissue integration and CAM vessel bleeding. Raising the preliminary incubation time to two weeks led to stable tissue formation and reduced cell migration *in ovo*. Conclusion: Sufficient cell delivery to the transplantation site is dependant on the preliminary incubation period rather than the initial cell load. Protocols intended for in vivo evaluation can be tested and optimised prior to their *in vivo* application using the HET-CAM assay.

Keywords: HET-CAM, cell transplantation, animal experiment

Lecture

A modified HET-CAM approach for biocompatibility testing of medical devices

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Introduction: The implantation of new biomedical devices into living animals without any previous toxicity or biocompatibility evaluation is possible based on current legislation. The HET-CAM (Hen Egg Test - Chorionallantoic Membrane) test system offers a partially immunodeficient, borderline *in vitro/in vivo* test system allowing the simulation of transplantation experiments to obtain biocompatibility data prior to animal testing. Material and Methods: A collagen type I/III scaffold designed for tissue regeneration was tested for angiogenetic properties and biocompatibility patterns. Biocompatibility was then altered by incubation in Acridine Orange/Ethidium Bromide and the test was repeated. Results: The native material demonstrated good biocompatibility patterns and a significant angiogenetic stimulus caused by the collagen scaffold material was observed. Altering biocompatibility patterns by incubation with Acridine Orange/Ethidium Bromide led to severe vessel thrombosis and a foreign body tissue response. Conclusion: CAM-Testing of biomaterials and tissue engineered products allows a selection of the most suitable biomaterial and an exclusion of improper materials from animal experiments, leading to refined testing procedures and a reduction of animal numbers required for biocompatibility testing.

Keywords: biocompatibility testing, HET-CAM, tissue engineering

Poster

Macroscopic evaluation of HET-CAM biomaterial testing: How reliable is macroscopical scoring without histology ?

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Introduction: The HET-CAM (Hen Egg Test - Chorionallantoic Membrane) test has originally been validated for toxicity and irritation studies and is under increasing attention for biomaterial evaluation purposes. The high vascularity of the CAM and the rapid development of connective tissue and vessel system offer an attractive environment for tissue reaction studies, which are often evaluated by macroscopical examination only.

Materials & methods: Various biodegradable scaffolds were applied onto the CAM on incubation day 7 and maintained *in ovo* for 3 days prior to digital documentation, macroscopical biocompatibility evaluation and subsequent histological analysis. A collagen sponge, two different Collagen Type I/III scaffolds (Chondro-Gide®, Bio-Gide®) and a Collagen Type II membrane (Chondrocell®) were tested. Macroscopic scoring criteria were embryo viability, vessel reactions, bleeding and infection. Histological criteria were angiogenesis induction and alteration of CAM structure.

Results: Collagen sponge: Macroscopic analysis demonstrated extreme rapid degradation, spontaneous bleedings in the surrounding of the implant and a vessel retraction from the implantation site. Histological analysis, in contrast, demonstrated an increase in blood vessel content compared to control CAMs. A foreign body tissue reaction was observed. Chondro-Gide®: Macroscopic evaluation showed excellent integration and biocompatibility patterns which were confirmed by histology. A tissue reaction was not observed and the scaffold showed excellent angiogenetic properties. Bio-Gide®: Macroscopic observation showed excellent integration and significant induction of angiogenesis, which was confirmed by histology. An

inflammatory infiltrate was observed. Chondrocell®: Macroscopic evaluation showed good integration of the biomaterial, which was confirmed by histology. Spontaneous bleedings at the implantation site as well as vessel retraction and altered vessel courses were observed macroscopically. Histological evaluation demonstrated good biocompatibility patterns, the absence of an inflammatory infiltrate and good angiogenetic properties.

Conclusions: Macroscopical scoring only partially correlated to histological evaluation: Tissue integration could be assessed with both methods. The impact of spontaneous bleedings and vessel path alteration was often overestimated and a foreign body tissue reaction could not be detected after macroscopical evaluation. HET-CAM biomaterial testing should therefore be combined with histological evaluation to receive the full force of expression of the _CAM model.

Keywords: HET-CAM, biomaterial testing

Poster

Invitrotrain: practical training courses on alternative (non-animal) test methods

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The Invitrotrain project covers the development, validation and – most importantly – the demonstration of *in vitro* methods for testing of chemicals and prediction of toxicity. The Free University of Berlin offers practical training of scientifically validated *in vitro* methods that have gained regulatory acceptance for specific purposes. The practical training courses focus on • skin models, skin corrosion and phototoxicity • acute eye irritation, • penetration models, • reproductive toxicology • ecotoxicology. All *in vitro* methods are hands-on laboratory exercise. The participants will perform the tests and evaluate the results according to Standard Operation Procedures or Invittox Protocols. The aims of the training courses are to provide the attendees with sufficient experience, so that they may apply the techniques to their own needs and to promote the use of *in vitro* alternative methods. The theoretical background of each test method will be introduced during seminars. Moreover, general aspects concerning the 3R's concept and validation studies will be addressed. The courses will take place biannually at the Institute for Pharmacy. The objectives of the Invitrotrain project are in line with the future EU policies on cosmetics and chemicals which call for the use of animal alternatives and new testing strategies. The project is sponsored by the European Commission.

Keywords: skin models, eye irritation, reproduction toxicology

Poster

Discussing serum-free cell culture – the product data base 2006

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The cultivation of cells *in vitro* is an essential tool for biomedical research and production purposes. For many tasks the supplementation of mammalian cell culture media with serum (-components) of animal origin remains still standard, providing for e.g. nutrition, shear protection, growth factors and cytokines. Because of undefined/varying composition, risk of contamination with prions/viruses/bacteria, the cost factor and also animal welfare considerations concerning the production of sera, the change to serum free alternatives is promoted by regulatory authorities, the industry and the research community in general. A growing number of alternatives exists for cell lines and primary cultures: chemically defined media, additives of non-serum origin, non-animal derived proteins and also optimized adaptation/sampling/processing protocols and production systems/bioreactors for serum-free usage of all scales. A regularly updated product guide (cur-

rently 2006) can be downloaded as PDF or HTML for free at: www.zet.or.at. Serum free media do not require supplementation with serum, nevertheless they may contain various undefined proteins and/or protein hydrolysates. Protein free media supporting growth and expression without the presence of proteins, protein-free media have eliminated many of the concerns regarding animal proteins in media supplementation. Some animal origin materials may remain in the protein-free product, such as amino acids. Chemically Defined Media All components have a known chemical structure, resulting in consistent product performance and the elimination of lot-to-lot performance variability.

Keywords: fetal calf serum, cell culture, serum free

Poster

Standardization o f the HET-CAM Assay for Biomedical Research: Analysis Software

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Incubated chicken eggs of early phases form an immunodeficient transition between *in vitro* and *in vivo* situations. The Chorionallantoic Membrane (CAM) - an extraembryonal membrane deriving from the fusion of chorion and allantois - provides an easily available, cheap and for certain applications validated testing environment. The test-system, originally conceived as alternative method for toxicity and irritation studies, has been suggested for tissue engineering tasks, biocompatibility testing and also as cell transplantation model. Evaluation of results often is performed just macroscopically by different members of working group. If digital documentation is performed, commercially available all-purpose software packages are used. Therefore interpretation/originality can be questioned, because images can be altered/modified easily. The authors use standardized biomedical software originally intended for other applications. Nevertheless, these programmes can be used/adapted for HET-CAM Bioassays thereby improving quality of data output and standardizing of HET-CAM Tests.

Keywords: HET-CAM, Software, Standardization

Lecture

HET-CAM model for melanom tumorgenicity studies

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The authors present a updated version of the Hen Egg Test-Chorioallantois Membrane (HET-CAM) angiogenesis test system which was originally designed and for certain applications validated for irritation/toxicology studies. New experimental setups and first data/results show suitability for innovative melanom tumor cell research: tumor formation, transmembrane malignant migration and possibilities of intervention/treatment *in ovo* and the potential consequences for conservative *in vivo* melanom models using rodent animal models/scientific animal welfare according to the 3Rs are discussed in detail.

Keywords: HET-CAM, melanom, tumor model, 3R

Poster

Carbon monoxide in angiogenesis research and the HET-CAM bioassay

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The Hen's Egg Test Chorioallantois Membrane (HET-CAM) angiogenesis system, originally developed for irritation studies, was used for evaluation of effects of exponation of early breeding stage chicken egg CAMs (up to incubtion day 10) to carbon monoxide (CO): quantitative/qualitative effects of low doses (50 - 500 ppm) upon development of vessels were evaluated. CO application of doses at this concentration are reported to effect cell cultures *in vitro*, modulating/triggering expression of heme oxygenase enzymes and therby effecting angiogenesis *in vivo*. The suitability of the *in ovo* HET-CAM system for e.g. angiogenesis/hypoxia but also CO-induced embryo toxicity testing is discussed.

Keywords: carbon monoxide, HET-CAM, angiogenesis, 3R

Lecture

[Difficulties and urgency of evaluating the genetic modifications of animals according to the three Rs principle]

Schwierigkeiten und Dringlichkeit der Evaluierung der gentechnischen Veränderung von Tieren nach den 3R-Prinzipien

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Als in den 90er Jahren das Verfahren der gentechnischen Modifikation eine breite Anwendung in der experimentellen biomedizinischen Forschung fand, sahen zahlreiche begeisterte Wissenschaftler in gentechnisch veränderten Versuchstieren den Schlüssel für die künftige tierexperimentelle Forschung sowie für die Eröffnung neuer vielversprechender Forschungsgebiete (z. B. Xenotransplantation). Trotzdem fehlen spezifische Richtlinien zur Haltung und Nutzung gentechnisch veränderter Versuchstiere und ihren speziellen Bedürfnissen und nur wenige Berichte über die Belastung ihres Wohlbefindens sind bis jetzt veröffentlicht worden. Insbesondere mangelt es an spezifischen Analysen auf der Basis der 3R-Prinzipien bezüglich der Implikationen der Verwendung von gentechnisch veränderten Tieren. Bei der Bewertung der gentechnischen Veränderung von Tieren in der Forschung haben sich zwei gegenläufige Strömungen entwickelt: Auf der einen Seite gibt es große Vorbehalte wegen der massiven Leidzufügung und dem großen „Tierverbrauch". Auf der anderen Seite wird in diesen Techniken ein enormes Potenzial für eine drastische Verminderung der gesamten Anzahl der Tierversuche gesehen (einige sprechen von der gentechnischen Veränderung als einer Alternativmethode). Unklarheiten bestehen noch in Bezug auf die angemessene Methode für eine Evaluierung der Forschungsbereiche. Die Vielfältigkeit dieser Bereiche und die Heterogenität der Zwecke der Verwendung gentechnisch veränderter Versuchstiere erschweren eine allgemeine Bewertung im Sinne der 3R-Prinzipien. Zusätzliche Probleme verursacht der große Mangel an spezifischen Daten über den Tierverbrauch und die Belastungen in den unterschiedlichen Phasen der Herstellung und Nutzung. In den meisten Tierversuchsstatistiken ist nämlich keine spezifische Kategorie für gentechnisch veränderte Tiere enthalten. In diesem Artikel werden zwei zentrale Fragen auch anhand einer Analyse empirischer Daten diskutiert: 1) ob die gentechnische Veränderung von Tieren einen Beitrag zur Verminderung der gesamten Zahl von Tierversuchen in der Forschung, bzw. zur Verbesserung der Effizienz der tierexperimentellen Forschung, leisten kann; 2) was für Konsequenzen für die gesamte Bewertung des Forschungsfeldes die Kollision zwischen den Prinzipien Reduction und Refinement hat, welche sich bei der Analyse vieler Anwendungen gentechnisch veränderter Tiere (z. B. bei Krankheitsmodellen) ergibt.

Keywords: 3R-Prinzipien, Ethik, gentechnische Veränderung, gentechnisch veränderte Tiere

Poster

Cell-growth promoting fractions originated from porcine blood clot

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Sera are generally obtained from drawn and collected whole blood from adult, calf or fetal animals. Following the natural clotting process, which may take several hours at 4 degree C, the blood consists of serum and blood clot containing: 95% of red blood cells, 5% platelets, less than 1% and numeorus amounts of fibrin strands. In comparison to a PRP (Platelet rich plasma) blood clot containin 4% of red blood cells, 95% pletlets and 1% of white cells. The specific cell-growth promoting components are the platelet derived growth factor (PDGF) and the transforming growth factor β (TGF β). Both of them are contained in the α granules of the platelets. Fibronectin and vitronectin are also the components of the PRP. They are the cell adhesion molecules found in plasma and fibrin itself. The experiments presented herein were aimed to isolate, characterise and to test *in vitro* on different cell cultures the growth promoting material from the swine blood clot. The swine blood was collected and allowed to form the clot. Afterward the whole content was centrifuged at 2500 RPM for 20 minutes and the supernatant (serum) was aspirated off. The sediment (»clot«) was quickly washed with the sterile buffered saline pH=6.9 for 20 minutes, and centifuged for 25 minutes at 2500 RPM for 25 minutes. The supernatant (Fraction I) was collected and frozen. To the remaining »clot« the PBS pH=7.2 was added and left for 1 hour at +4oC. After the centrifugation of the suspension at 2500 RPM for 25 minuts, the supernatant (Fraction II) was collected and frozen. To the sediment (»clot«) the PBS pH = 7.4 was than added for 18 hours (Fraction III) . All the fractions were sterilised by 0.2 membrane filtration. The content was analysed by PAG-SDS electrophoresis. The cell growth promotion/inhibition activity in the comparison to the SR-2.055P (Serum replacement based on porcine ocular fluid) and FCS (Fetal calf serum) was tested on the Chicken embryonal fibroblasts, WISH, HAC-3/T2 (Human amniotic cell lines), PLA-2 (Adult pig kidney cell line), Bovine intestinal epithelial cell line, WiREF (Wistar rat embrional fibroblastoid cell line) and CaCo-2(Colon cancer carcinoma cell line). The results of the experiments shows: (1) The attachment and growth of primary cultures (Chicken embryonal fibroblasts) is not affected after the use of procine blood clot fractions (2) Fractions (I-IV) shows the growth promotion, but different according to the group of cells used in the test. (3) The strongest growth enhancement was found when transformed cells (WiREF, CaCo-2) were tested. (4) The optimal content was 8-10% in Eagle's medium. In this range up to 95% value of the SR-2.55P could be obtained.

Keywords: swine, blood clot, growth factors, cell growth

Poster

Questioning the ethical justification of primate experiments by launching a campaign for a legal ban

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Primates are sentient beings with high cognitive abilities. It is increasingly recognized by the public, scientists and politicians that their use in experiments causes extreme suffering. By laying down the animal welfare into the German constitution in 2002 Germany took an important step towards protecting animals for ethical reasons, which is now a governmental obligation. But until today it is not clear what this means concerning animal experimentation and which experiments are no longer ethically justifyable under this legislation. In October 2004 People for Animal Rights Germany have started the campaign "It's my life" to stop primate experiments in Germany. The campaign includes informing the public and working on juridical and political level. We also want to raise the question whether primate experiments are ethically justifyable under Article 20a of the German constitution. Our aim is to show that they are not – and therefore convince politicians to make use

of their governmental obligation by amending the German Animal Welfare Law to outlaw primate experiments. In some EU countries (Austria, Sweden, Netherlands) experiments at least on great apes have been made illegal. In Germany no experiments on great apes have been conducted since 1991. Our campaign wants to see all primate experiments included in a future ban and is also seen in the light of the currently ongoing amendment of the Directive 86/609.

Keywords: primates, ban, German Animal Welfare Law

Lecture

Human organoids for immunogenicity and immunotoxicity testing

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Preclinical evaluation of biological drug candidates, e.g. antibodies, vaccines and growth factors, demands novel predictive cell based assays. Species specificity of those tests is crucial for valid efficacy and side effect data. Three-dimensional tissue culture of the respective species, e.g. human, seems to be mandatory. In contrast to chemical drugs, any injection of biologics induce a light to severe immune response. In case of vaccines this particular response is the most wanted. For therapeutical antibodies for example, immunogenicity is a no go criteria. We will present concepts of organoid culture, to address vaccine efficacy and immunogenicity of biologics. Complex co-cultures of human dendritic cells and lymphocytes were performed in miniaturised perfusion cell culture devices. Cytokine patterns, histological analyses and cell imaging are used to evaluate functionality and responsiveness of these tissue cultures. First attempts were made to multiplex, miniaturise and automatise the systems for later high content. Process performance was evaluated for robustness, consistency and standardisation.

Keywords: cell based assay, immunogenicity, lymphatic organoid, perfusion culture

Lecture

A new high potent freezing medium closes the last gap in the "serum free" cell culture technique

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In the recently completed project sponsored by FFVFF Zurich, we adapted several cell lines frequently used by biotechnologists and molecular biologists to chemically fully defined culture media. We followed the growth behaviour before and during the adaptation phase. After a stringent quality control and authentication, the cell lines are now available for the scientific community by the European Collection of Cell Cultures (ECACC). Cells cultured without FBS (fetal bovine serum) are in general more sensitive to chemical agents and mechanical stress. The proteins in FBS, consisting mainly of albumins, create a balanced viscosity in the cells' environment and bind added reagents, as well as toxic metabolites. These facts are especially important in the process of cryo-conservation as it is often done using a mixture of culture medium, FBS and DMSO as anti-freeze agent. The main part of our presentation deals with the evaluation of a freezing protocol designed for cells cultured in absence of FBS. Therefore we developed a new chemically fully defined cryo-medium with Pluronic F68™ as active ingredient. Using FILOCETH as freezing medium with Pluronic F68™ medium we demonstrate the recovery of living cells after thawing of a cryo-conserved cell sample being factor 1.5 higher as compared to the standard FBS/DMSO mixture. We also show the influence of different cooling rates on the recovery rate of frozen cells.

Keywords: cryoprotectant, chemically fully defined cell freezing medium, chemically fully defined medium

Poster

Establishment of a functional cell culture model of the pig small intestine

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Cell culture models play a pivotal role in laboratory research of various fields. Appart of existance of many various cell lines, there is a substantial lack of good intestinal cell culture models. In spite of the fact, that human colon tumorigenic cell lines, like Caco-2, are widely used as a model of a small intestine, such cell lines do not represent the normal small intestine of the man. It is accepted, that pig is much better animal model than rodents in human research , therefore we have established a functional cell model of an adult pig small intestine, consistent of two pig intestinal epithelial cell lines (PSI and CLAB) and macrophages from the pig peripheral blood (PoMac). All newly established cell lines were were spontaneously transformed to the continous cell line and characterised by morphological, immunochemical and functional characteristics. Results obtained proved, that the established functional cell model of the pig small intestine can be successfully us ed in various studies involving the small intestinal tract, as well as in studies of local or systemic immunity.

Keywords: cell line, functional cell model, small intestine, pig

Poster

On the status of citizens' rights to information in German-speaking countries

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Germany, Austria and Switzerland represent the taillights of Europe with regard to citizens' information rights. In many countries worldwide, citizens can inform themselves on how the state spends their tax money. This is not so in the three alpine republics.

This form of access to information has enjoyed its longest tradition in Sweden. Already in 1766, the citizens attained the right to study official documents based on the freedom of the press. This right was anchored in the Swedish constitution. What is called „Offentlighetsprincipen" in Sweden is now internationally known as „*Freedom of Information*" or „*The Principle of Public Access*".

In the USA this right was extended by Bill Clinton in 1996, by provisions for information requests via Internet.

It has been investigated where in the world this principle has been introduced, where it is planned and where it is absent (Harvard-Banisar-Study, 2002). In Germany, a first attempt was started on 1.1.2006 on the federal level, which must still be tested from the animal protection side. From Austria we have not yet heard of any kind of political approach towards this subject.

In Switzerland, it is often not even recognized as a problem by the politicians. The secretiveness of the officials in Switzerland is so pronounced that members of the cantonal committees for animal experiments may not even engage experts for certain subjects as this would breach official secrecy requirements.

Who is protected by this official secrecy?

Is it the privacy of the scientist who could otherwise perhaps not defend him- or herself against public hostility?

Is it to protect the intellectual property of the scientists?

Does the agency itself need to be protected?

Regulatory agencies in many ways simply choose to believe in science. At least this is less work than evaluating the truth and probability content of academic claims. The committees for animal experiments are not in a position to do this because of the official secrecy requirements. It is easier to leave the public in doubt about what experiments are planned and what arguments speak for or against them. Fear of (ill-)in-

formed laypersons may also play a role, even though sufficient well-educated specialists are now available who can read and interpret applications and evaluate them with regard to animal protection aspects.

Animal protection requires that at least those animal experiments that are financed with public funds should be accepted by the public. Starting at a medium level of severity they should be discussed in a suitable manner prior to approval.

Keywords: freedom of information, regulatory agencies, committees for animal experiments, tax money

Lecture

Safety in the cell culture laboratory

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Safety is a prerequisite in every workplace. Identifying and evaluating risks, and taking appropriate action to avoid or minimize them, are the basis on which safety is built in any laboratory.

Risk assessment in cell culture laboratories demands special awareness of potential hazards associated with the biological materials that are handled, and of the specific physical and chemical hazards related to cell and tissue culture work.

The risks associated with cultured cells are dependent upon the species and the mode of origin (primary cultures or cell lines), and the procedures and techniques applied. Thus, the biosafety level of the cultured cells have to be defined. Cell cultures presenting the highest risks, for example, are those of human and primate origin. As a consequence, the cultured cells should be handled at a containment level in compliance with national biosafety regulations. Other potential biological hazards that should be considered involve biological components of culture media (serum, tissue extracts, growth supplements) and/or culture products, e.g. in eukaryotic expression systems, virus propagation or vaccine production. In addition, techniques and protocols to generate genetically modified cells, for example in mutagenesis, *in vitro* transformation, cell transfection, or cell hybridization can be hazardous.

The design and management of a cell culture laboratory are important considerations with regard to safety. Containment is the most obvious means of reducing risk from cell cultures that demands the use of microbiological safety cabinets or laminar flow hoods. For most cell lines, the appropriate biosafety level is *Level 2* requiring a Class II microbiological safety cabinet.

The cell culture laboratory is not a particularly dangerous place to work with regard to chemical hazard, although general safety standards should always be maintained to protect workers and the environment.

Physical hazards in cell and tissue culture are directly related to the maintenance and storage of cultured cells. These include "heat" (e.g. autoclave and dry-heat oven), pressure and/or vacuum in sterile filtration, and extreme "cold" in handling liquid nitrogen at freezing, storage, and thawing of cells.

Finally, appropriate safety measures in waste disposal should always be applied.

Keywords: risk assessment, biosafety level, biohazard

Lecture

Assessment of applicability and tolerability of drugs and excipients on lung cell models without animal testing

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Introduction: Processes of drug development are growing more complex and time consuming. Therefore, smart concepts should be integrated into the formulation development prior to clinical studies to streamline the development in terms of costs and risks. In-vitro cell culture studies instead of animal studies prove to be a fast and cost-effective tool to assess the applicability of a formulation in the early development stage. This paper summarizes results with airway and lung cells as tool for an excipient and formulation screening. Methods: Calu-3 cells were cultivated in Minimum Essen-

tial Medium (MEM) with Earl's Salts and L- supplemented with 10% FCS. Primary cell were isolated as described in Steimer et al. 2005. For toxicity and permeability studies cells were seeded on Transwell clear® filter inserts. The cell cultures were standardized by verifying the TransEpithelial Electrical Resistance (TEER) and transport markers for high and low permeable transport. The transport characteristics of drug substances and the toxic effect of formulations and excipients have been investigated. Results: Different formulations with the same drug (e.g. Cyclosporin A) showed toxic effect on cell monolayers dependent on the used excipients as well as concentrations of the drug and/or excipient. Additionally the transport mechanism of drug substances and the influence of formulation could be investigated. Conclusions: Based on the cell results data, we conclude that cell culture models, are a useful tool to study permeation as well as irritation or toxic effects of drugs, formulations and/or common excipients selected to be used for inhalation. The tests offer the possibility to investigate potential effects of the drugs and formulations in fast and cost effective way before further development efforts and money are invested in more expensive development steps or preclinical and clinical evaluation of drug formulations with a markedly decrease of animal studies.

References: Steimer, A., Haltner, E. et. Al., *J. Pharm. Res.*, submitted.

Keywords: drug development, inhalation, toxicity, permeability, transport mechanism, aerosols

Lecture

Expansion of the embryonic stem cell test: Differentiation into neural cells

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The embryonic stem cell test (EST) is a validated *in vitro* assay that has been established to classify compounds with respect to their embryotoxic potential. The current experimental procedure involves differentiation of murine embryonic stem cells (D3) into contracting cardiomyocytes. However, potentially embryotoxic drugs may effect primarily other tissues than the myocardium. Consequently, this consideration prompted us to expand the EST to other major target tissues. Here, we present a protocol for differentiation of murine embryonic stem cells into neurones designed with special regard to the testing of chemicals. This modified protocol is based on a monolayer differentiation procedure and offers the advantage of a reproducible development of neural cells in a comparatively short time. The differentiation of D3 cells into neural cells was characterised by analysis of neurone-specific marker gene expression using flow cytometry. In addition, the developing neurones were examined by immunofluorescence staining using neurone-specific antibodies. As a result, we were able to define neurone-specific molecular endpoints for the detection of chemical effects on embryonic development. Preliminary chemical testing revealed different sensitivities between neural cells and cardiomyocytes for a limited number of test chemicals. The expansion of the EST to more than one target tissue will considerably improve the accuracy of this predictive screen by preventing false negative results. Moreover, the differentiation of murine embryonic stem cells into neurones might prove to be a useful tool in developmental neurotoxicity testing as well as in pharmacological testing.

Keywords: in vitro, embryotoxicity, embryonic stem cell test, differentiation, neurons, developmental neurotoxicity

Lecture

Towards eliminating the use of animals for regulatory required vaccine quality control

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Traditionally, regulatory required vaccine quality control relies heavily on the use of laboratory animals. Both batch testing for safety and testing for potency is based on animal models and quite often these models include procedures that induce severe pain and distress. Thus, it is estimated that for instance potency testing of tetanus and diphtheria toxoid vaccines requires more than one million animals per year world-wide and as a challenge procedure with tetanus toxin is part of the protocol, half of the animals is expected to die from tetanus. These figures have urged the development of 3R methods. In this presentation an overview will be given of some major 3Rs achievements and breakthroughs that ultimately will allow batch quality control without the use of laboratory animals. These include the replacement of challenge procedures by serological methods, the reduction of numbers of animals by single dose instead of multi-dose testing, and the developments in the area of *in vitro* models and physicochemical procedures. Central in progress towards elimination of animal use is the acceptance of the consistency approach. The redline in the consistency approach is that a new batch of vaccine is no longer seen as a unique product but as only one of a series of batches produced from the same seed lot. As a consequence, a batch of vaccine produced shares many of the characteristics of the previous batches that were produced from the same seed lot. This allows for a new strategy of vaccine quality control, giving emphasis to aspects such as in process testing, the implementation of GMP principles and to quality assurance. These approaches particularly rely on non-animal test models. An outline will be given of a protocol for the consistency approach and potentials and possible pitfalls will be discussed.

Keywords: vaccine, quality control, toxoid, consistency, in vitro

Poster

Measuring nociception by fMRI in anesthetised animals

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There is a demand for novel analgesics to provide relief for different types of pain. During development of new analgesics traditional pa in tests are performed measuring behavioural reactions of awake animals which consequently suffer from these painful stimuli. A solution could come from measuring responses to painful stimuli in anesthetized animals non-invasively by functional magnetic resonance imaging (fMRI). This method provides highly resolved objective functional information on the processing of nociceptive stimuli throughout the whole brain as has already been demonstrated in human pain studies. Consequently, this method could also improve objective measurements of modulatory effects of analgesics. Moreover, optimized data-analysis strategies can help to reduce the number of experiments needed to obtain a representative activatin maps e.g. for different analgesics. We established such a fMRI testing system in anesthetized rats using a mild noxious heat stimulation applied to rat hindpaws. Because the testing is applied to animals under anaesthesia with mild stimulation (temperature: 34 - 45°C, suitable for humans too) we are minimizing the stress for the animal. Moreover, we were able to 1) obtain reliable objective information of different pain competent structures along the pain pathway 2) by applying modern image processing and data analysis techniques we were able to obtain representative group average results at a minimal number of experiments. By doing so we could define the degree of pain suppression of different conventional analgesics. Such a system can further be applied for investigating (chronic) pain processes. This would open a new avenue for research on pain chronification and it may contribute to the evaluation of novel more specific analgesics intended to inhibit or even reverse chronic pain.

Keywords: fMRI, rat, human, computer, image processing

Poster

Investigating mitochondrial toxicity of nucleoside reverse transcriptase inhibitors and ethidium bromide *in ovo*

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Mitochondrial dysfunction is a major toxicity of nucleoside reverse transcriptase inhibitors (NRTIs), used for treatment of human immunodeficiency virus (HIV) infection. NRTIs induce mitochondrial dysfunction mainly through inhibition of mitochondrial (mt) DNA synthesis which could lead to a wide range of severe adverse effects. Lactic acidosis and hepatic steatosis are increasingly recognized in NRTI treated patients. Since at present no clinically relevant *in vitro* model exists to investigate mitochondrial toxicity, *in vivo* studies are mostly used to confirm preliminary *in vitro* data. There is a need for developing non-animal models to detect mitochondrial toxicity induced by NRTIs. The aim of this study was to establish an *in ovo* model for the investigation of mitochondrial toxicity. Incubated hens' eggs were treated with the NRTIs zalcitabine (ddC), didanosine (ddI), stavudine (d4T), and zidovudine (AZT) between days 5 and 9 by air cell administration. Ethidium bromide (EtBr), an agent known to deplete mtDNA in cell cultures, was used as positive control. Mitochondrial toxicity was investigated at day 11 of incubation, when the embryo's nervous system is not yet completely established. Mitochondrial function was analysed by measuring blood lactate levels and ATP content in erythrocytes. mtDNA content in relation to nDNA content was determined in embryos' livers using a novel dual colour real-time PCR based assay. Treatment with AZT, ddI, ddC, and EtBr resulted in significant increase in blood lactate levels. A significant decrease of ATP content was only observed for AZT and EtBr. In the liver a significant depletion of mtDNA was found for ddI, ddC, and EtBr but not for AZT, a weak inhibitor of mtDNA synthesis. d4T did not induce significant changes on the parameters tested. The results are in good agreement with those of other published data from *in vitro* and *in vivo* studies. The *in ovo* model is a simple, sensitive, and rapidly responding alternative model to animal testing, applicable for studying mitochondrial toxicity of NRTIs and other agents.

Keywords: in ovo, chick embryo, mitochondrial toxicity, nucleoside reverse transcriptase inhibitor, ethidium bromide, alternative to animal testing

Lecture

Increased reproducibility in toxicity testing using Epidermal Skin Test 1000

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The reliability of skin corrosivity testing using epidermal models was shown already with different reconstituted systems. The successful classification in this field is required for the more subtle task of correct identification of substances with skin irritative properties. To facilitate correct classification in those studies the skinmodels were reconstituted of neonatal foreskin keratinocytes as it is widely accepted that using those cells enhance reproducibility. Here we present data on the reproducible and reliable classification of all twelve reference compounds of OECD TG 431: "*In Vitro* Skin Corrosion: Human Skin Model Test" using Epidermal Skin Test 1000 (EST-1000). The results are obtained in a blinded multicenter trial fulfilling all the criteria to be peer reviewed according to the ECVAM validation strategies. To support our data the proper morphology of EST-1000 is demonstrated by an extensive histological and immunohistochemical characterization. Furthermore the intact barrier function is confirmed by showing the resistance against several solutions of detergents over a long period and for different batches. Additionally, we present promising data using this adult Epidermal Skin Test in testing of irritative as well as phototoxic properties. With these data we demonstrate the adaption of this skin model to the actual purpose. In respect to animal testing this adaptability is one major advantage of *in vitro* skin models.

Keywords: skin, epidermal model, skin corrosion, skin irritation, reconstructed skin

Lecture

A novel *in vitro* cell model of muco-ciliated human airway epithelium for long term toxicity testing

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Most of the *in vitro* cell models for long term testing of chemicals suffer of at least two shortcomings: 1)The failure of reproducing the *in vivo* physiological characteristics of the corresponding tissues, such is the case for the immortalised cell lines. 2) A limited shelf-life, for example, the freshly established primary cell cultures. Our company has developed and commercialises a novel *in vitro* cell model of the human airway epithelium which is free of these limitations. The epithelium is not only morphologically and functionally differentiated; it can also remain at a homeostatic state for more than six months. Indeed, under the electronic microscope, the typical ultrastructures of the human airway epithelium, such as the tight junctions, the cilia, the basal cells, the mucuous cells, could be observed. The epithelium is resistant when measured by trans-epithelial /cm^2. The ionΩelectrical resistance (TEER): the mean value is about 450 channels like the sodium channel and chloride channel are fully functional and respond normally to their specific inhibitors and activators when measured in modified Ussing chambers. Moreover, the epithelial cells react to pro-inflammatory mediators such as TNF-α in a physiological manner: the amount of secreted interleukin-8 (a marker of inflammatory reaction of the airway epithelium) increased significantly after TNF-stimulation, and then decreased gradually to the basal level after 5 days of recovery. Remarkably, the epithelium has a strong capacity of regeneration after mechanical or chemical injuries. Furthermore, our *in vitro* cell model, when generated with cells isolated from patients suffering of genetic diseases (cystic fibrosis, primary ciliary dyskinesia), reproduces the pathological phenotypes of the disease. The fidelity and the longevity of our *in vitro* human airway epithelium cell model make it a new valuable tool for the long term toxicity testing of molecules.

Keywords: primary cell culture, airway epithelium, long term toxicity testing

Lecture

Knowledge of the human genome (total DNA sequence) as an opportunity to build better drugs and concomitantly reduce the need for animal experimentation

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Although laudable and to some extent cost-effective for the pharmaceutical industry, the latter will not set about large-scale reduction of animal testing unless major financial incentives and viable alternatives are forthcoming. A detailed knowledge of the Human genome and post-genomic systems integration offer concrete potential to achieve both. For the first time in the history of the pharmaceutical industry, the scientific community has access to an accurate 'Parts-List' of all the drug targets in the Human body. However, because this translates into some 20 billion distinct drug-binding sites, each needing to be assessed '*in silico*', the task is highly computationally intensive. Encouragingly, the last decade has also witnessed significant advances in computing power. As in other scientific disciplines, the biomedical sciences and drug development must become increasingly driven by computation as part of efforts to more accuratel y prediction Adverse Drug Effects prior to entering animal testing and clinical trials. Both humans and animals will benefit directly from such approaches. Concrete examples will be drawn from the recent failure of Vioxx TM, the >27,000 heart attacks and sudden cardiac deaths registered by the FDA in association with Vioxx TM during its stay on the market and the $10 billion (USD) negative impact on the Market Capitalisation of the Merck Corporation as proof of concept. A new era of enhanced target selectivity (better drugs) can run hand-in-hand with short term reduction of animal testing due to what is termed: 'Early Cull', namely killing potentially deleterious compounds well in advance of animal and clinical testing. Successful delivery of such can also result is large-scale economies of both cost and time during drug development.

Keywords: enhanced target selectivity, proteomics, biocomputing, in silico modelling, drug screening, adverse side effects

Poster

Progress in alternative concepts in Turkey

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Awareness of animal suffering and possible replacements of animal experiments have been receiving great attention by communities around the world. The three Rs of Russell and Burch introduced during the 50s put profound influence on scientific communities. With the directive "The Council Directive for the Protection of Vertebrae Animals Used for Experimental and other Scientific Purposes -" in 1986, the EU countries are required to adopt new regulations. Besides governing the Member States, the directive 86/609/EEC also influenced indirectly other European countries including Turkey. Interactions between Turkish researchers with European counterparts played a crucial role in this sense. Turkey, an EU candidate country, made significant progresses recently as far as laws and regulations on animal experimentation. Turkish Parliaments voted the new Animal Rights Act on the 24th of June 2004. In this sense, directives concerning animal experiments, ethics committees, and lab animal husbandry have been prepared and under review. The directive of the Ministry of Agriculture has already proclaimed a new directive on laboratory animal medicine so that the use of animal has been regulated by legal acts. The Scientific and Technical Research Council of Turkey, the main grant provider for scientific studies in Turkey, no longer grants fund for animal experimentations without approval from an ethic committee. Alternatives to animal experimentations concept is fairly a new concept in Turkey. Ethic committees now have been questioning if possibly the animal experiments can be replaced or reduced to some degree with cell culture or with lower vertebrae. For example, in Gulhane Medical Academy (GATA), the nematod *Caenorhabditis* elegans has been used in aging and genetic studies. There are no formal lectures or education on alternatives yet. However, awarness among researchers and lab animal scientists are the driving forces at this point. There has been an awaking among Turkish colleagues for alternative methods. However, investment on education of investigators and development of alternative methods are crucially important. In addition, transferring experience of European colleagues is equally important. The bond between international and European organisations such as ECVAM and ICLAS should be strengthened.

Keywords: alternatives, animal experiments, Turkey, directive, law regulations

Poster

Herbal cosmetic ingredients – protective effects assessed *in vitro* and proved *in vivo*

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Active ingredients of herbal origin became a regular part of cosmetic products, especially in formulations intended for consumers with sensitive or dry skin, with the aim to improve skin condition and appearance. Herbal ingredients are reported to promote physiological functions of the skin and may offer a balanced complex of health effects as moisturising, free radical scavenging, calming and anti-inflammatory, improving skin elasticity, anti-aging, healing sunburn or chemical induced irritation. The aim of the study was to assess the significance of addition of selected herbal ingredients, used in formulations of cleaning products in order to minimise possible adverse irritative effects of tensides, e.g. Sodium Dodecyl Sulfate (SDS). The protective effects of selected active ingredients were tested *in vitro* in the cell culture of 3T3 fibroblasts and in the human reconstructed skin model (EpiDerm ™) and subseqently evaluated *in vivo* by testing in a group of volunteers by means of closed epicutaneous patch test according to COLIPA Guidelines. Protective effects against SDS cytotoxicity were demonstrated in the cell culture in case of a number of natural substances, e.g. Green Tea, Aloe Vera, Pronalen Sunlife, Pronalen Cereal, Sea Silk, Pronalen Sensitive Skin and Chamomile. The effects of the most promising substances were confirmed in the 3D human skin model. Results from the *in vitro* systems were compared to results obtained in an experimental study comprising epicutaneous test in a

group of volunteers. Although all the selected herbal substances, in accordance with results obtained *in vitro*, exhibited protective effect against SDS-induced skin irritation in human volunteers, the highest degree of protection *in vivo* was proved for Pronalen Sensitive Skin and Chamomile. The *in vitro* test systems were found to be a useful tool for screening of biological effects and became a valuable part of regular safety and efficacy testing of cometics, employed before confirmatory testing in human volunteers.

Keywords: herbal ingredients, cell culture, 3D skin model, cytotoxicity, epicutaneous test, irritation

Poster

Facilitating the implementation of best practice education through access to and training in alternatives

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Access to alternatives plays a crucial role in familiarising teachers with the diversity and quality of tools that are available to support best practice life science education. Equally important is training that allows for a more detailed exploration of specific alternative tools and approaches. To meet the need for access to and training in alternatives, InterNICHE maintains an Alternatives Loan System, and organises training seminars across the world to provide expert training. The Alternatives Loan System is an evolving library of alternatives available for free loan worldwide. It was established during 2001-2002 and includes over 100 CD-ROMs, videos, simulators and training mannekins, chosen for their pedagogical value and potential to replace common dissections and animal experiments. Borrowers include teachers, students, animal ethics committees, government ministries, organisations and campaigners in over 40 countries. The facility has serviced over 200 loans, comprising over 4000 usages of individual alternatives. As a tool for facilitating implementation, the value of the Loan System is indicated by significant teacher use and the high number and wide geographical range of loans, subsequent purchase and implementation of products, direct replacement of harmful animal use, and providing an international resource for campaigners. Small-scale 'micro-Loan Systems' have been established in Brazil, Russia, Ukraine, India and Japan. Since its inception in 1988, InterNICHE has organised demonstrations and training at annual conferences and dedicated training seminars. The alternatives used are selected for their relevance to specific national and cultural realities in order to maximise the opportunities for replacement. Using the Alternatives Loan System and the skills of local teacher trainers, over 400 university teachers were trained in alternatives and animal welfare during August-September 2004 at seminars in over 10 cities across India. This project was organised by InterNICHE in conjunction with the World Society for the Protection of Animals (WSPA) and many committed local organisations, and was the first of its kind worldwide that provided training at a national level. The network is planning further alternatives training for 2006, including across Latin America, the Middle East and North Africa.

Keywords: alternatives, animals, best practice, education, India, InterNICHE, library, students, teachers, training

Lecture

Internationalising alternatives in higher education

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InterNICHE has been working internationally to promote and implement alternatives in higher education for 18 years, facilitating the replacement of harmful animal use and building a broad network with contacts in over 50 countries. From the InterNICHE experience, successful international work requires qualities and practices from organisations that include: a bold and positive vision, and an awareness of the links between issues; a commitment to pro-actively catalyse sustainable change and create win-win solutions; the design of organisational structures conducive to participatory

democracy, alliance building and the organic growth of the network; the practice of solidarity and support for local initiatives rather than empire building; and the provision of resources and training for action and capacity building. The presentation will draw on examples of InterNICHE projects such as the production and multi-language translations of printed, video, and website resources; the Alternatives Loan System for trial of software, mannekins and simulators anywhere in the world; the international Humane Education Award for local development and implementation of alternatives, including freeware; support for student conscientious objectors; and conferences, outreach visits, and training in alternatives for teachers. An overview of the international momentum towards full replacement of harmful animal use in education will be presented. The challenges met within such work will also be explored, and suggestions of how to overcome them will be given.

Keywords: alternatives, animals, education, freeware, grant, internet, InterNICHE, library, students, training, translation

Poster

Physicochemical profile of testosterone and caffeine and permeability across human skin *in vitro*

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The objective of this work is to define a practical sequence scheme for a fast and reasonable development of semisolid formulations without animal testing. The development of new topical formulation is depending on drug and skin specific properties. The way from active compound to a proper semisolid formulation is a challenge. For this purpose four active compounds were cho-sen from the corticosteroid group: Betamethasone valerate, Clobetasol propionate, Hydrocorti-sone and Mometasone furoate. Two OECD-markers for *in vitro* percutaneous absorption: Caf-feine and Testosterone were taken additionally as controls. Three different semisolid formulations with an active agent concentration of 0.1 % were used as delivery system (amphiphilic cream DAC, hydrophobic anhydrous wool-fat ointment DAB and hydrophilic carbomer hydrogel DAB). Physicochemical profiling and the *in vitro* release and permeability profile across human skin of the model drugs testosterone and caffeine will be here presented. The transport studies showed that permeation from formulations with testosterone and caffeine is dependent on formulation type. For both analyzed substances we could see the highest re-lease rate from the hydrogel. For the lipophilic compound, testosterone, the maximum absorp-tion rate was observed from the hydrophilic hydrogel. In contrast, the results of the *in vitro* permeation studies for the hydrophilic caffeine from three different formulations show the highest permeation rate from the hydrophobic ointment, which shows the lowest release rate. For both test substances the lowest transport through the analyzed human membranes was seen from the amphiphilic cream. The observed release profiles of testosterone and caffeine show that the release rate of the drug from the formulation to the skin surface is not always the rate-limiting step in the overall drug diffusion process. We hope to make some contributions to the reduction of the animal tests in the development of formulations for topical application by using meaningful *in vitro* alternatives and physicochemical methods.

Keywords: semisolid formulations, alternative methods, physicochemical metohods, skin absorption in vitro

Lecture

Ecotoxicological tests and recombinant luminescent microbial models in toxicity studies: contribution to 3Rs

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Due to industrial development and the rapid growth of human population, the role of chemicals in the everyday life has considerably increased. Ecotoxicology addresses the environmental hazards of xenobiotics already accumulated in the environment and also of newly produced chemicals. The political awareness on knowledge on human safety and environ-

mental effects of chemicals is reflected by the new EC chemical policy REACH. Both, the development of new chemicals and environmental protection require toxicity testing that up to now implies the usage of a very large number of laboratory animals. The majority of the so far proposed *in vitro* tests – although greatly reducing the number of experimental animals – involve the use of cell and tissue cultures derived from vertebrate species. The role of the whole non-animal organisms (e.g. bacteria, crustaceans, protozoa, plants, yeasts) in human health risk assessment, especially for regulatory purposes, is still relatively small. Although many assays using non-animals as test organisms, e.g. *Vibrio fischeri* bioluminescence inhibition assay, Daphnia mortality assay, algal growth inhibition test etc., have been validated and even accepted on regulatory level (OECD, ISO), these tests have mainly been used in ecotoxicity testing. The application of ecotoxicological tests (e.g., luminescent bacteria, protozoa, crustaceans) at least at the early screening stage of all areas of toxicological research should be seriously considered, as this could save a lot of time, money, manpower and lives of experimental animals. The single non-specific toxicity tests, although unreplacable in evaluation of integral toxic effects, are not able to identify mechanisms of toxicity. However, competent interprepation of a test battery data may lead to a better understanding of toxic action. Different non-vertebrate tests have shown to be sensitive and of reasonable predictive power for a number of organic and inorganic chemicals and even nanoparticles – new emerging toxicants. Last but not least, specific microbial assays may be set up by genetically modifying bacteria (prokaryotic models) or yeasts (eukaryotic models). It is possible to incorporate into these organisms special reporter elements which synthesis will be triggered as a response to specified target bioavailable chemical(s) and thus reporting on their transfer into the cell and resulting biological effects *in vivo*. Moreover, the microbial models may be chosen according to their medical significance, amenability to genetic manipulations, physiological simplicity and ease of handling. Recombinant luminescent bacteria that increase their light production in response to bioavailable heavy metals and phenols have been constructed and used as powerful tools in analysis of the bioavailability of these compounds in complex matrices.

Keywords: Vibrio fischeri, protozoa, crustaceans, recombinant bacterial sensors, bioavailability, REACH, nanoparticles, 3Rs

Poster

Inhouse validation of a screening test for the determination of acute fish toxicity using embryos of zebra fish

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Acute fish toxicity is an important endpoint for ecotoxicological hazard identification and assessment of chemicals. It has been shown that fish embryo testing has the potential to serve as a replacement for *in vivo* testing on fish. In order to build up methodologies that can be used as screening tools during substance development or to prioritize *in vivo* toxicity testing, a screening protocol using embryos of zebra fish was developed and validated in our laboratory. The screening test was conducted in 24-well plates using one fertilized zebra fish embryo per well. The embryos were maintained in our ecotoxicology facilities. More than 40 test substances with known acute fish toxicity covering a wide range of chemistry, including agrochemicals were used to validate this test system. The substances were either dissolved in water, or acetone used for highly lipophilic compounds as a solvent, respectively. Four different dose groups covering a concentration range of three to four orders of magnitudes were used with 20 embryos each. Additionally, another 20 embryos at a time served as solvent (water, where necessary acetone) and positive (3,4-dichloraniline) controls. All test compounds have been tested three times at minimum. LC_{50} values were estimated from defined lethal endpoints as described in the literature and compared to acute fish LC_{50} values. The results derived from testing on fish embryos using this screening protocol correlated well with the acute toxicity in fish and showed good reproducibility. Therefore, this test system can at least be used as a tool for screening and prioritizing purposes. Additionally, we pursue the development, validation and regulatory acceptance of alternative methods (3R concept of Russell and Burch, 1959), which are highly needed regarding the new EU Chemicals Regulation REACH and the Cosmetic Directive as well as for other regulatory purposes. Thus, further efforts are undertaken to contribute to a full validation of the fish embryo test that may become a replacement for acute tests using adult fish.

Keywords: fish embryo, acute fish toxicity, screening, alternative methods, validation

Poster

Development and evaluation of a robust, common protocols for reconstructed human skin models: *In vitro* skin irritation/corrosion testing

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Due to the increased need for information on the safety of chemicals (driven mainly by new the chemicals policy of the European Commission (REACH)) a lot of attention is paid to development and validation of *in vitro* methods replacing or reducing the use of test animals. Reconstructed human skin models offer a possibility to satisfy regulatory requirements for testing of local toxicity and at the same they contribute to the 3R approach. For *in vitro* skin irritation and corrosion testing several test assays have been successfully developed in the past applying diverse reconstructed human epidermal (RHE) models. However, since several RHE models disappeared from the market after validation (and with them the validated assays), a common test protocols, applicable to all well developed and standardised RHE models had to be developed. For skin corrosion and acute phototoxicity, the usefulness of a common protocol has been already proven (Liebsch et al., 1997; Liebsch et al., 1999). Recently an attempt has been made to develop a \"common skin irritation protocol\" for EpiDerm and EPISKIN RHE models (Cotovio et al., 2005; Kandárová et al., 2005). This protocol is currently evaluated in an ECVAM Skin Irritation Validation Study. To show that the \"common protocol concept\" works ZEBET has in 2004 performed two additional studies employing the SkinEthic RHE model. First, in a co-operation with BASF AG (Germany) and SafePharm (UK) we applied the common skin corrosion protocol of OECD TG 431. Than in a co-operation with SkinEthic Laboratories (France) and SCHERING AG Germany) we applied the \"common skin irritation\" protocol to SkinEthic RHE. After minimal adaptations specific for SkinEthic RHE model almost identical results compared to EpiDerm and EPISKIN were obtained in both assays. In the above mentioned studies the reliability of the common protocol and similarity between three reconstructed human epidermal models have been evaluated and confirmed. Minor, probably model-related differences in prediction have been observed. However, taken into account large variability of *in vivo* responses, the above mentioned RHE models and protocols can provide reliable prediction of irritation and corrosion potential of chemicals, that are concordant between laboratories and over time.

Keywords: skin irritation, skin corrosion, in vitro, common protocol, validation, RHE models

Poster

Humane teaching methods demonstrate efficacy in veterinary education

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Animal use resulting in harm or death has historically played an integral role in veterinary education, in disciplines such as surgery, physiology, biochemistry, anatomy, pharmacology, and parasitology. However, the last decade has seen a rapid increase in the availability of non-harmful alternatives, such as computer simulations, high quality videos, 'ethically-sourced cadavers' such as those from animals euthanased for medical reasons, preserved specimens, models and surgical simulators, non-invasive self-experimentation and supervised clinical experiences. However, experience has shown that many veterinary faculty remain opposed to such teaching methods, usually citing teaching efficacy as their main concern. Consequently studies were reviewed comparing learning outcomes generated by non-harmful teaching methods with those achieved by harmful animal use. Of ten studies from 1989 to 2000, nine assessed surgical training – historically the discipline involving greatest harmful animal use. 30% (3/10) demonstrated superior learning outcomes using more humane alternatives. 60% (6/10) demonstrated equivalent learning outcomes, and only one study demonstrated inferior learning outcomes. Eleven additional studies in which comparison with harmful animal use did not occur illustrated other benefits of humane teaching methods, namely; time and cost savings, increased repeatability and flexibility of use, customization of the laboratory experience, more active learning, facilitation

of autonomous and life-long learning, improved attitudes towards computers and alternatives to animal use, and increased employer perception of computer literacy. The results indicate that veterinary educators can best serve their students and animals, while minimizing financial and time burdens upon their faculties, by introducing well-designed teaching methods not reliant upon harmful animal use.

Keywords: alternative, animal experiment, education, training, veterinarian

Poster

Animal carcinogenicity studies: Implications for the REACH System

[Full paper to be published 2006 in *Alternatives to Laboratory Animals 34*, suppl., in press.]

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The 2001 European Commission proposal for the Registration, Evaluation and Authorisation of Chemicals (REACH) aims to improve public and environmental health by assessing the toxicity of, and restricting exposure to, potentially toxic chemicals. The greatest benefits are expected to accrue from decreased cancer incidences. Hence the accurate identification of chemical carcinogens must be a top priority for the REACH system. Due to a paucity of human clinical data, the identification of potential human carcinogens has conventionally relied on animal tests. However, our survey of the US Environmental Protection Agency's (EPA's) toxic chemicals database revealed that, for a majority of the chemicals of greatest public health concern (93/160, i.e. 58.1%), the EPA found animal carcinogenicity data to be inadequate to support classifications of probable human carcinogen or non-carcinogen. A wide variety of species were used, with rodents predominating; a wide variety of routes of administration were used; and a particularly wide variety of organ systems were affected. These factors raise serious biological obstacles that render the meaningful extrapolation to humans profoundly difficult. Furthermore, that International Agency for Research on Cancer assessments for the same chemicals are significantly different, indicates that the true human predictivity of animal carcinogenicity data is even poorer than is indicated by the EPA figures alone. Consequently, we propose the replacement of animal carcinogenicity bioassays with a tiered combination of non-animal assays, which can be expected to yield a weight-of-evidence characterisation of carcinogenic risk with superior human predictivity. Additional advantages include substantial savings of financial, human and animal resources, and potentially greater insights into mechanisms of carcinogenicity.

Keywords: REACH, alternative, animal experiment, animal test, bioassay, cancer prevention, carcinogenicity, chemical classification, chemical safety, computer simulation, in vitro, risk assessment

Poster

Animal carcinogenicity studies: 1) Poor human predictivity

[Full paper published 2006 in *Alternatives to Laboratory Animals 34*, 19-27.]

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The regulation of human exposures to potentially carcinogenic chemicals constitutes society's most important use of animal carcinogenicity data. Environmental contaminants of greatest U.S. concern are listed in the Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS) chemicals database. However, of the 160 IRIS chemicals lacking even limited human exposure data but possessing animal data as of January 1, 2004, we found that in most cases (58.1%; 93/160) the EPA considered animal carcinogenicity data inadequate to support a classification of

probable human carcinogen or non-carcinogen. For the 128 chemicals with human or animal data also assessed by the World Health Organization's International Agency for Research on Cancer (IARC), human carcinogenicity classifications were compatible with EPA classifications only for those 17 having at least limited human data (p=0.5896). For those 111 primarily reliant on animal data, the EPA was much likelier than the IARC to assign carcinogenicity classifications indicative of greater human risk (p<0.0001). The IARC is a leading international authority on carcinogenicity assessments, and its significantly different human carcinogenicity classifications of identical chemicals indicate that: (i) in the absence of significant human data the EPA is over-reliant on animal carcinogenicity data, (ii) as a result, the EPA tends to over-predict carcinogenic risk, and (iii) the true predictivity for human carcinogenicity of animal data is even poorer than indicated by EPA figures alone. EPA policy erroneously assuming that tumours in animals are indicative of human carcinogenicity is implicated as a primary cause.

Keywords: animal experiment, animal test, bioassay, cancer prevention, carcinogenicity, chemical classification, chemical safety, risk assessment

Poster

Animal carcinogenicity studies: 2) Obstacles to extrapolation of data to humans

[Full paper published 2006 in Alternatives to Laboratory Animals 34, 29-38.]

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Due to limited human exposure data, risk classification and the consequent regulation of exposures to potential carcinogens has conventionally relied mainly upon animal tests. However, several investigations have revealed animal carcinogenicity data to be lacking in human predictivity. To investigate the reasons, we surveyed the 160 chemicals possessing animal but not human exposure data within the U.S. Environmental Protection Agency chemicals database that had received human carcinogenicity assessments by January 1, 2004. We found a wide variety of species used, with rodents predominating; a wide variety of routes of administration used, and a particularly wide variety of organ systems affected. The likely causes of the poor human predictivity of rodent carcinogenicity bioassays include (i) the profound discordance of bioassay results between rodent species, strains and genders, and further, between rodents and human beings; (ii) the variable yet substantial stresses caused by handling and restraint, and the stressful routes of administration common to carcinogenicity bioassays, and their effects on hormonal regulation, immune status and carcinogenesis predisposition; (iii) differences in rates of absorption and transport mechanisms between test routes of administration and other important human routes of exposure; (iv) the considerable variability of organ systems in response to carcinogenic insults, between and within species; and (v) the predisposition of chronic high dose bioassays towards false positive results, due to the overwhelming of physiological defences, and the unnatural elevation of cell division rates during ad libitum feeding studies. Such factors render attempts to accurately extrapolate human carcinogenic hazards from animal data profoundly difficult.

Keywords: animal experiment, animal test, bioassay, cancer prevention, carcinogenicity, chemical classification, chemical safety, extrapolation, risk assessment

Poster

Animal carcinogenicity studies: 3) Alternatives to the bioassay

[Full paper very recently published 2006 in Alternatives to Laboratory Animals 34 (1), 39-48.]

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Conventional animal carcinogenicity tests take around three years to design, conduct and interpret. Consequently, only a tiny fraction of the thousands of industrial chemicals in use have so far been tested for carcinogenicity. Despite the cost of hundreds of millions of dollars, millions of skilled personnel hours, and millions of animal lives, several investigations have revealed animal carcinogenicity data to lacking in human specificity (ability to identify human non-carcinogens), which severely limits its human predictivity. Causes include the scientific inadequacies of many carcinogenicity bioassays, and numerous serious biological obstacles, which render attempts to accurately extrapolate human carcinogenic hazards from animal data profoundly difficult. Proposed modifications to conventional bioassays have included the elimination of mice as a second species, the use of genetically-altered or neonatal mice, decreased study durations, initiation-promotion models, greater incorporation of toxicokinetic and toxicodynamic assessments, structure-activity relationship (computerised) systems, *in vitro* assays, cDNA microarrays for detecting genetic expression changes, limited human clinical trials, and epidemiological research. Potential advantages of non-animal assays when compared to bioassays include superior human specificity results, substantially reduced timeframes, and greatly reduced demands on financial, personnel and animal resources. Inexplicably, however, regulatory agencies have been frustratingly slow to adopt alternative protocols. In order to decrease cancer losses to society, a substantial redirection of resources away from excessively slow and resource-intensive rodent bioassays, into the further development and implementation of non-animal assays, is both strongly justified and urgently required.

Keywords: alternative, animal experiment, animal test, bioassay, cancer prevention, carcinogenicity, chemical classification, chemical safety, computer simulation, in vitro, risk assessment

Poster

Chimpanzee research: 1) Questionable contributions to biomedical knowledge

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Biomedical research on captive chimpanzees incurs substantial animal welfare, ecological, ethical and financial costs. Advocates of such research claim these costs are outweighed by substantial advancements in biomedical knowledge. To assess the accuracy of such claims we examined the disciplines investigated in 749 studies of captive chimpanzees or chimpanzee tissues conducted from 1995-2004. 48.5% (363/749) were biological experiments conducted in nine disciplines, with cognition/neuroanatomy/neurology (36.6%, 133/363) and behaviour/communication (20.7%, 75/363) arising most frequently. 41.5% (311/749) were investigations of 30 viral groups, with hepatitis C virus and human immunodeficiency virus, which both comprised 31.2% (97/311) of all virology experiments, arising most frequently. Therapeutic investigations comprised 3.5% (26/749) of all chimpanzee experiments, of which 61.5% (16/26) explored the pharmacological properties of various compounds. Other investigations included the testing of surgical techniques or prostheses, anaesthesiology and toxicology experiments. Investigations of eight parasitic species comprised 3.1% (23/749) of all chimpanzee experiments, of which the most frequent were the malaria protozoa *Plasmodium falciparum* and *P. ovale* (26.1%, 6/23), the roundworm *Onchocerca volvulus* (21.7%, 5/23), and the flatworm

Schistosoma mansoni (17.4%, 4/23). Other diseases and miscellaneous experiments comprised 3.5% (26/749) of all chimpanzee experiments when combined, of which the most frequent were investigations of laboratory/husbandry techniques (42.3%, 11/26) and endotoxaemia (30.1%, 8/26). Radiation studies were also conducted, and four other diseases were also investigated, namely benign prostatic hyperplasia, Creutzfeldt-Jakob disease, gastrointestinal bacteriology (*Bacillus thuringiensis*), and tuberculosis (*Mycobacterium tuberculosis*). To assess the value of these 749 chimpanzee studies, we randomly selected 100 and determined the frequency with which they were cited by other published papers. Citations were not available for four studies, however, 49.0% (47/96) of the remainder were not cited by any other papers. Research of lesser value is not even published. These results indicate that the majority of chimpanzee research generates data of very little use, and contributes very little to the advancement of biomedical knowledge.

Keywords: animal experiment, animal research, chimpanzee, bonobo, Pan troglodytes, Pan paniscus

Poster

Chimpanzee research: 2) Lack of efficacy in combating human disease

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Advocates of increased expenditure on chimpanzee research claim their genetic similarities to humans enables this research to make critical contributions to combating human diseases. To assess the validity of such claims, we randomly examined 100 studies of captive chimpanzees from a population of 749 studies conducted between 1995 and 2004. Citations were not available for 4 chimpanzee studies. 49.0% of the remainder (47/96) were not cited by any other papers, demonstrating minimal contribution towards the advancement of biomedical knowledge generally. One chimpanzee study was cited only by a paper for which no abstract was available, and was discarded. Of the 95 remaining chimpanzee studies, 36.8% (35/95) were cited only by 116 papers that clearly did not describe well developed diagnostic or therapeutic methods for human diseases. Instead, abstracts focused primarily on a surprising array of non-human species, including a large variety of primates; or on human subjects in relation to a variety of biological disciplines other than pathology; or on examinations of the aetiological or other aspects of human diseases. Only 14.7% (14/95) of applicable chimpanzee studies were cited by a total of 27 papers that appeared to describe well developed prophylactic, diagnostic or therapeutic methods for combating human diseases. However, detailed examination of these 27 human medical papers revealed that *in vitro* studies, human clinical and epidemiological studies, molecular assays and methods, and genomic studies, contributed most to their development. 63.0% (17/27) of these medical papers were found to be wide-ranging reviews of 26-300 (median 104) references, to which the cited chimpanzee study made only a small contribution. Duplication of human outcomes, inconsistency with other human or primate data, and other causes resulted in the absence of any chimpanzee study able to demonstrate an essential contribution, or, in most cases, a significant contribution of any kind, towards the development of the human medical method described.

Keywords: animal experiment, animal research, chimpanzee, bonobo, Pan troglodytes, Pan paniscus

Poster

Chimpanzee research: 3) The necessity of a ban

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The advanced sensory, cognitive, social and communicative abilities of chimpanzees also confer upon them a profound ability to suffer when captured from the wild or born into unnatural captive environments, and when subsequently subjected to confinement, social disruption, and involuntary participation in potentially harmful biomedical research. Advocates justify such research based on the crucial contributions they claim it has made towards the advancement of biomedical knowledge, and, in particular, towards combating major human diseases. However, our systematic review of 749 biomedical studies of captive chimpanzees conducted during a recent decade revealed that almost half were not cited by any subsequent papers within the comprehensive bibliographic databases examined, suggesting minimal contribution towards the advancement of biomedical knowledge generally. Given that research of lesser significance is not published at all, these results are of considerable concern. Furthermore, closer examination of a subset of 100 randomly-selected chimpanzee studies failed to identify any that made an essential contribution, or, in a disturbing majority of cases, a significant contribution of any kind, towards papers describing well-developed prophylactic, diagnostic or therapeutic methods for combating human diseases, including major diseases such as AIDS, hepatitis and cancer. Consequently, we therefore call for, and believe it eminently reasonable to call for, the banning of biomedical research on captive chimpanzees in those remaining countries, notably the US, that continue to conduct it.

Keywords: animal experiment, animal research, chimpanzee, bonobo, Pan troglodytes, Pan paniscus

Poster

Sensitivity of the zebrafish embryo (*danio rerio*) to pesticides: Visible effects versus cholin- and carboxylesterase enzyme biomarkers of insecticide exposure

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The acute zebra fish embryo test is an accepted bioassay to assess the toxicity of waste water which may be used for the replacement of testing with adult fish. It is also suggested for chemical hazard assessment although only a few groups of substances have been studied yet. Specifically acting substances like neurotoxic insecticides pose a potentially hazard for non-target fish. To establish whether the proposed zebra fish embryo test protocol and the inhibition of cholinesterases (acetylcholinesterase EC 3.1.1.7, propionylcholinesterase EC 3.1.1.8) and carboxylesterase (EC 3.1.1.1)enzymes can be used in a similar fashion for risk assessment, two types of experiments were conducted. Visual ffects of exposure to the organophosphate paraoxon-methyl after 24 and 48 h in the zebra fish embryo test system were analysed with the use of an inverse microscope(rate of mortality, developmental disturbances, heart rate and others). The inhibition to cholinesterases and carboxylesteras e was also measured. Enzyme inhibition as a biomarker was about 70 times more sensitive than the effects in the zebra fish embryo test with an IC_{50} below 1.2 µmol compared to an EC_{50} of 91 µmol. Significant overt effects could only be seen at concentrations at which already 80% of the activities of the different esterases were inhibited.

Keywords: cholinesterase, carboxylesterase, zebra fish embryo test, insecticide, bi omarker, paraoxon-methyl

Poster

Trans-Epithelial Electrical Resistance monitoring in perfusion culture is a reliable indicator of monolayer integrity and barrier function

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Trans-Epithelial Electrical Resistance (TEER) is a useful parameter to quantify barrier function in endothelial and epithelial cells and has been demonstrated to be a sensitive endpoint for determining the toxicity of compounds to epithelial cells *in vitro*. The aim of this study was to establish TEER measurement to monitor barrier function of epithelial cells under continuous perfusion conditions. Epithelial cell lines, exhibiting different degrees of monolayer tightness, were cultivated on microporous growth supports in a perfusion apparatus (EpiFlow®) with an implemented TEER measuring unit. Confluent cell monolayers were intoxicated with various toxic concentrations of CdCl2 (0-15 µM). The toxic influence on cell layer integrity was monitored via TEER measurement and additionally controlled by measuring lactate dehydrogenase (LDH) activity in the out-flowing medium. During formation of the epithelial monolayer, TEER increased to a steady state value. CdCl2 exposure resulted in a gradual collapse of TEER, which correlated with an increased LDH release. TEER is a sensitive, simple, non-invasive online measuring tool of cell monolayer integrity. TEER monitoring increases the usability of perfusion technology for the determination of time resolved toxicity.

Keywords: TEER, EpiFlow, perfusion culture, epithelial cells

Poster

Non-invasive measurement of adrenocortical activity in male and female rats

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Rats are widely used as animal models for human diseases. However, due to their relatively small body size, blood sampling is difficult, invasive and thereby might seriously interfere with endocrine functions and animal welfare aspects. Therefore a non-invasive technique to monitor stress hormones in these animals is highly desired. Our study aimed to gain information on corticosterone metabolism and to validate a 5α-pregnane-3β,11β,21-triol-20-one enzyme immunoassay (EIA) to monitor corticosterone metabolites (CM) in faecal samples of laboratory rats. Six rats of each sex were administered 2.4 MBq of ^{3}H-corticosterone intravenously and three of each sex received the labeled steroid per os. Subsequently, all voided excreta were collected for five days. Peak concentrations in urine appeared after 1.7 ± 0.6 h and after 6.0 ± 3.5 h in males and females, respectively. In faeces, excretion maxima were observed after 14.7 ± 2.4 h in both sexes. In principal the route and delay for both administration methods was the same, except a delay of peak concentration in urine (4.5 ± 2.1 h) in per os administered males. About 75% of the recovered CM was found in the faeces. Using high performance liquid chromatography (HPLC) substantial information about the biochemical characteristics of faecal ^{3}H-CM was revealed and differences between the sexes were found. In both sexes, corticosterone was heavily metabolized. While males showed only minor variations in their CM patterns, those of females differed largely between individuals. To validate the EIA, we conducted an ACTH challenge test, a dexamethasone suppression test and investigated effects of the diurnal variation (DV) of glucocorticoids, using six male and six female rats each. Our results demonstrated that pharmacological stimulation, suppression and the DV of adrenocortical activity were reflected accurately by means of CM measurement in faeces. Therefore, our find-

ings confirm the suitability of this EIA to non-invasively monitor adrenocortical activity in rats of both sexes, which can open new perspectives for biomedical and pharmacological investigations as well as animal welfare related issues.

This study was funded by the Austrian Federal Ministry for Education, Science and Culture (GZ 80.102/2-BrGT/2004).

Keywords: non-invasive, stress, glucocorticoids, rats

Lecture

Animal experiments in biomedical research. An evaluation of the clinical relevance of approved animal experimental projects: No evident implementation in human medicine within more than 10 years

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Retrospective evaluation of the ethical review process for animal experimentation has been demanded for years. One main aspect in evaluating this system is in investigating whether the prospective cost-benefit-analysis that served as the basis for granting licenses for procedures can be regarded realistic when compared to the actual outcome of the scientific research. Previous investigations have indicated that the distress for the animals has commonly been underestimated in applications for granting a license, and that the majority of investigated research projects failed to deliver the envisioned specific scientific results. In the present study, we looked at the clinical significance of research projects in those cases where applicants suggested a concrete benefit for the cure of human diseases. Only those projects were taken into account where previous studies had shown that the results of the animal research applied had confirmed the hypotheses of the researchers. A retrospective citation analysis for 12 years was performed and the results were unambiguous: after analysis of more than 1000 scientific articles: It has become evident that none of the 17 analysed applications at three German universities led to any new therapies or had any clinical impact. According to Directive 86/609 (Art. 12/2), painful animal experimentation need to be "of sufficient importance for meeting the essential needs of man or animal" to be licensed. The results of our study verify that in specific cases the practice of licensing of animal research in Germany fails completely. With regard to the "benefit"-factor, authorities have to apply much more stringent criteria when certifying the importance of the proposed research. For this "cost"-factor, as indicated in previous studies, the basis for prospective assessment of pain, suffering, and distress has to be objectified. The authorities hardly ever raise any serious doubts about the significance of the proposed research. Our study confirms the need to change perspectives, approaches, and questions so they can be addressed by non-animal methods. This notion should be enforced by the authorities. From an animal welfare perspective, the main paradigm of the licensing practice has to be turned upside down: when there is any question about the relevance of an at least painful animal research the decision in doubt should be for the animal.

Keywords: animal experiments, ethical review, ethical evaluation, ethical committees, cost-benefit-analysis, citation analysis, clinical relevance

Poster

Study of the clinical relevance of 51 applications on animal experiments in biomedical research.

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In Germany, according to the German Animal Welfare Act, scientists must provide prior to undertaking an animal experiment an ethical and scientific justification in their applications to the licensing authority. In such justifications reference is made to lacking knowledge with regard to development of human diseases or the need of better and even new therapies for humans. Basis of the present study is the literature research, based on applications of biomedical study groups of three universities in Bavaria (Germany) between 1991 and 1993 (Lindl et al. *ALTEX, 18*, 171-178, 2001). Here we show the aim of each research (n = 51), the animal model, the reasons had been given to the choice of the animal model and whether the research has led or not led to a publication found in DIMDI or PUBMED. These applications have been classified according to their publications as successful in the animal model (Lindl et al. 2001). We investigated here in greater detail the frequency of citations, the course of citations, and the question in which type of research the primary citations have been taken up: In subsequent animal based studies, in In-vitro-studies, in review articles or in clinical studies. The criterion we applied was whether the scientists succeeded to reach the goal that they postulated in their applications: To contribute to new therapies or to gain results of direct clinical impact. The outcome was unambiguous: even though 97 clinically orientated publications in which the above mentioned publications were cited could be tracked (8 % of all citations), only in 4 publications a direct correlation between the results from animal experiments and observations in humans could be noted (0,3 %). But even in these 4 cases the hypotheses that had been verified successfully in the animal experiment failed in any respect. The implications of our findings may lead to demands concerning improvement of the licensing practice in Germany. Acknowledgement First results of this study were presented as Poster at the 12th Congress on Alternatives to Animal Experiments at the university of Linz, Austria and at the Fifth World Congress on Alternatives & Animal Use, Berlin, August 2005. The Poster was honoured with the Poster-award \"LINZ 2004\" We gratefully thank the Austrian \"Zentrum für Ersatz- und Ergänzungsmethoden zu Tierversuchen\" (zet) and the \"VIER PFOTEN-Stiftung für Tierschutz\" for the award. We also thank Dr. Ingrid Weichenmeier and Dr. Birgit Lewandowski for their helpful investigations into literature.

Keywords: animal experiments, ethical review, ethical evaluation, ethical Postercommittees, citation analysis, clinical relevance

Poster

[Vascularized liver test system as an alternative to animal tests] Vaskularisiertes Lebertestsystem als Alternative zu Tierversuchen

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Die Entwicklung von Alternativen zu Tierversuchen ist essentiell, um neue Wirkstoffe früh in der Entwicklungsphase an humanen Zellen untersuchen zu können und somit eine bessere Übertragbarkeit von Ergebnissen zu gewährleisten. Die Leber spielt eine zentrale Rolle im Stoffwechsel. Deshalb sind Leberzellen (Hepatozyten) bzw. das gesamte Organ von besonderem Interesse als Analysesystem für Substanzen und Wirkstoffe sowie ihre Metaboliten. Existierende *in vitro* Testsysteme sind sehr artifiziell, die Zellen werden nicht unter physiologischen Bedingungen kultiviert und zeigen deshalb nur einen kleinen Anteil der biologischen Reaktionen im Körper. Wir entwickeln ein Testsystem auf der Basis einer vaskularisierten porcinen Matrix, die in einem Bioreaktormodul die physiologische Versorgung von Hepatozyten in Co-Kultur mit Endothelzellen ermöglicht. Im ersten Schritt wird ein Stück porcines Jejunum mit erhaltenem Gefäßsystem (mit arteriellem Zufluß und venösem Abfluß) chemisch azellularisiert (1). Das Gefäßsystem dieser Ma-

trix wird mit porcinen Endothelzellen aus der Leber rebesiedelt. Die Besiedlung erfolgt unter pulsatilem Fluß für 7 Tage bei 37°C und 5% CO^2. Nach einer Woche Kultur wird die Co-Kultur mit Hepatozyten gestartet.Die Zellen werden immunhistologisch (z.B. CD31, CKLP 34, Anti Human Hepatocyte) untersucht und ihre Vitalität (Live Dead Assay) überprüft. Es werden Harnstoff- und Albuminsynthese, LDH-Aktivität und Galaktoseabbau der Hepatozyten analysiert. Endothelzellen konnten erfolgreich in das Gefäßsystem integriert werden. Nach zweiwöchiger Kultur sind sie vital und exprimieren endothelzellspezifische Marker. Die Kultur von Hepatozyten auf der azellulären, vaskularisierten Matrix zeigt bei einem Kulturzeitraum von 2 Wochen gute Ergebnisse für das Wachstum der Zellen und den Erhalt leberspezifischer Funktionen. Die in das Matrixlumen aufgebrachten Leberzellen sind vital, exprimieren leberspezifische Marker und beginnen in die Matrix einzuwandern. Die Hepatozyten bilden auf der Matrix zwei bis drei Zelllagen in typischer Lebermorphologie. Unser Ziel ist die Entwicklung eines vaskularisierten Lebermoduls für verschiedene Applikationen. Durch die Entwicklung eines Lebertestsystems auf Basis der vaskularisierten Matrix wäre es erstmals möglich Wirkstoffe arteriell zu applizieren und venös zu analysieren. Ein derartiges Modell kann als Testsystem zur Identifizierung von potentiellen Wirkstoffen oder toxischen Metaboliten herangezogen werden und so zur Reduzierung oder zum Ersatz von Tierversuchen beitragen.
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Keywords: Vaskularisiertes Testsystem, Leber, Co-Kultur

Poster

Gene expression profiling of an *in vitro* tissue model for cartilage destruction

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An rheumatoid arthritis (RA) *in vitro* pannus tissue model was developed to study molecular processes of cartilage destruction by synovial fibroblasts. Human articular chondrocytes were cultured in alginate beads for 2 weeks to form neo-cartilage. Immortalized RA (RASF) and normal (NDSF) human synovial fibroblasts were cultured for 48 h and supernatants were collected. This conditioned synovial fibroblast medium was used to stimulate neo-cartilage alginate beads for 48h. Normal cell culture medium served as controls. Gene expression profiling of stimulated alginate beads was performed with Affymetrix oligonucleotide microarrays. In addition, RASF and NDSF were treated with the frequently used anti-rheumatic drugs methotrexate, prednisolone or diclofenac and used for subsequent microarray gene expression profiling. The expression profiles of selected genes were verified by semi- quantitative real-time PCR and by ELISA. Neo-cartilage alginate beads stimulated with RASF conditioned medium showed differential expression of 150 genes compared to beads cultured in the presence of NDSF conditioned medium. Most of these genes are associated with cartilage formation and destruction (e.g. cartilage oligomeric matrix protein, cathepsin S), inflammation and immune response (e.g. interleukins: IL1alpha and IL8) as well as cell adhesion (e.g. beta 8-integrin, vascular cell adhesion molecule 1 (VCAM1)) known from RA. In RASF, the cytostatic drug methotrexate results in the reversion of the RA-related expression profile of genes associated with growth and apoptosis (e.g. IGFB3), whereas the glucocorticoid prednisolone reverted the RA-related profile of genes that are known from inflammation (e.g. IL1beta and IL8). The non-steroidal anti-inflammatory drug diclofenac showed no effects on the RA-related gene expression. Mediators produced by RASF promote destruction of articular cartilage as known from rheumatoid arthritis pannus tissue. Moreover, RA-related mediators and gene expression profiles of RASF can be reverted to normal by common anti-rheumatic drugs. Therefore, RASF and neo-cartilage alginate beads may represent a useful *in vitro* RA pannus model for screening of putative anti-rheumatic compounds.

Keywords: cartilage, rheumatoid arthritis, in vitro model, microarray, inflammation, drug screening

Lecture

[The question of right to life in animal ethics]

Ein Lebensrecht für Tiere – ethisch zu rechtfertigen?

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Innerhalb der Tierschutzethik ist wohl keine Frage zu finden, die so kontrovers diskutiert wird wie die des Lebensrechts. Selbst unter „tierfreundlichen" Philosophen gibt es den größten Dissens, unter welchen Voraussetzungen eine Tiertötung ethisch gerechtfertigt werden kann. Die aktuelle Diskussion um die Tötungsfrage resultiert aus der Speziesismus-Kritik der frühen 1970er Jahre. Peter Singer (*1946) gehörte damals zu den ersten, die den üblichen Umgang mit Mensch und Tier als speziesistisch, d.h. als ohne plausibles Differenzierungskriterium die Vertreter der eigenen Spezies (Homo sapiens) bevorzugend, „entlarvt" hat. In Weiterführung eines Gedankens von Jeremy Bentham (1748-1832), der erstmals die Leidensfähigkeit als moralisch entscheidende Eigenschaft beschrieb, bemüht sich die Ethik seither darum, neben dem Menschen auch alle leidensfähigen Tiere zu integrieren (sog. Pathozentrische Ethik). Die Speziesismus-Kritiker argumentieren im Sinne einer Beweislastumkehr; was de m Menschen Recht ist, soll – bis zum Nachweis fehlender Voraussetzungen – den Tieren billig sein. Die aus der Speziesismus-Kritik entstandenen Argumente zur Tötung bauen auf dem (fragwürdigen) Postulat auf, eine Tötung sei deswegen unmoralisch, weil sie gegen das Interesse der Person verstoße, weiterzuleben. In Anlehnung an Leonard Nelson (1882-1927) wird so aus Tierrechtler- und Veganerkreisen für ein Lebensrecht aller Tiere argumentiert. Peter Singer meint jedoch, dass das Argument, solche Tiere nicht zu töten, die weiterleben wollen, nur auf diejenigen Tiere anwendbar sei, die diesen Gedanken tatsächlich zu denken im Stande sind, also ein reales „Interesse am Weiterleben" haben; er nennt als Beispiele für eine solche „Präferenz" u.a. Affen, Hunde und Katzen. Bestärkt wird seine Einschätzung durch Tom Regan (*1938), der – als Kritiker der Singer'schen Methodik – zu einer ganz ähnlichen Teilung des Tierreichs in der Tötungsfrage gelangt. Die Anerkennung eines Lebensrechts b ei kognitiv höher entwickelten Arten wurde dann z.B. von Ursula Wolf (*1951) und Jean-Claude Wolf (*1953) jeweils im Rahmen paternalistischer Interpretation des erwähnten Grundgedankens wieder auf alle bewusst-empfindungsfähigen Tiere ausgedehnt. Von früheren Philosophen wurde im Anschluss an die antike Philosophie geschlussfolgert, dass Tiertötungen angst- und schmerzlos durchgeführt werden müssen, jedoch kein Lebensrecht der Tiere hergeleitet werden könne. – Die beiden Argumentationsgruppen werden vorgestellt und ihre Stärken und Schwächen beleuchtet.

Keywords: animal killing, animal welfare, bioethics, professional ethics

Poster

Characterisation of two im mortalized human corneal cell lines used for building a new organotypic corneal equivalent

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Recently, a new organotypic, three-dimensional corneal equivalent based on SV40-immortalized human cell lines was reconstructed by embedding keratocytes (HCK) in a collagen matrix, layering epithelial cells (HCE) on top and endothelial cells (EC) below (Zorn- Kruppa et al., 2005). The aim of this work was to evaluate this cornea model as a tool for eye irritation testing, permeation studies and wound healing processes by characterising EC and HKC. The main attribute of EC is to build a functional monolayer, while the phenotype of HCK as well as their ability to transform into a fibrotic phenotype (myofibroblasts) is known to be crucial in the wound healing process (Matsuda et al., 1973). Five culture mediums were tested in order to evaluate the effect of serum and calcium concentration on cellular response. For EC characterization cells were tested by fluorescence activated cell sorting (FACS) and immunocytochemistry (ICC). Antibodies for the nuclear markers Ki-67 and p27KIP1 were used to evaluate cellular proliferation and contact inhibition re-

spectively. Primary human corneal endothelial cells were used as positive control. In all the mediums tested EC failed to build a constant monolayer. The expression of Ki-67 did not show a significant decrease after confluence, while low expression of p27KIP1 confirmed the absence of contact inhibition. In conclusion, growth conditions of the EC cells still have to be optimized. The HCK-phenotype was assessed in culture, with and without stimulation through TGF-β, as well as in cryosections of the stromal matrix. The expression of α- smooth muscle actin (α-SMA), a specific marker for the myofibroblast phenotype, was detected by ICC. Primary human fibroblasts were used as positive control. As result, HCK did only show significant _-SMA expression after TGF-β stimulation. In conclusion, the immortalized HCK proved to be the keratocyte phenotype which can be stimulated to differentiate into myfibroblast form. Hence, the HCK are suitable for the assessment of eye injury and wound healing.

Keywords: cornea model, immortalized keratocytes, immortalized endothelial cells, immunocytochemistry

Lecture

Veterinary training without the use of laboratory animals – an example from Norway

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This presentation describes the steps taken by a Norwegian veterinary student to successfully complete her veterinary education using alternatives to laboratory animals. This included the use of computer simulations, student self-experiments in physiology, dissections on waste material from the pathology department and naturally dead animals, and surgical training through beneficial procedures in veterinary clinics. The presentation offers an example of how a veterinary student can complete her education with commitment to the principle of \"First, do no harm\", and addresses how this principle relates to the use of animal experiments. Describing practical solutions as well as discussing the reasons for an approach without the harmful use of animals, the author argues that this approach can and should be implemented as the standard method of education for veterinary students.

Keywords: alternatives, animals, animal experimentation, clinical, dissection, ethically-sourced, InterNICHE, veterinary education

Poster

The InterNICHE policy on the use of animals and alternatives in education

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The InterNICHE 'Policy on the Use of Animals and Alternatives in Education' is a comprehensive document in 10 sections that addresses all aspects of work with animals and alternatives in life science education. The Policy presents guidelines to ensure effective and fully ethical acquisition of knowledge and skills. It includes a definition of alternatives in education and of harm, and presents individual policies on dissection, the sourcing of animal cadavers and tissue, work with live animals for clinical skills and surgery training, and ethical field studies. It also addresses the use of animals for the production of alternatives themselves. While the ideal 'replacement alternative' is defined as 'non-animal' within the 3Rs philosophy of Russell and Burch (1959), the Policy highlights a shortcoming of the 3Rs approach for education. Not only is there a requirement for some students to work with animals, animal tissue and clinical procedures in their education, there is wide spread evidence of the ability to fully meet all teaching objectives in ways that are neutral or beneficial to individual animals and that do not involve animal experimentation or killing. As well as non-animal learning tools like multimedia computer simulation, digital video, training models and mannekins,

replacement alternatives also include the use of ethically-sourced animal cadavers for dissection and skills training, and apprenticeship into clinical practice with animal patients. A definition of 'ethically-sourced', and of ethical educational opportunities within clinical work, are included in the Policy which demonstrates the possibilities for full replacement of harmful animal use in education.

Keywords: 3Rs, alternatives, animals, education

Poster

Statistical aspects of experimental animal studies

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Experimental animal studies are performed to gain further knowledge about biological processes. They have to be justified and properly designed to come to sound conclusions, to gain as much reliable information as possible and to keep the number of animals as small as possible. Sound knowledge of study design aspects (e.g. parallel groups, factorial designs, block designs, …), statistical analyses strategies and sample size calculation should be a matter of course for study protocols, not only in clinical trials involving humans, but also in animal trials. The importance of these points and frequent problems/misunderstandings are presented and discussed with a special focus on real problems of submitted studies to animal ethics committees in Austria.

Keywords: study design, sample size calculation

Poster

Biosensor for environmental and bacterial toxins based on immobilized fish chromatophores

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The design, operation and performances of a biosensor based on immobilized living chromatophores isolated from Betta splendens Siamese fighting fish are presented in this paper. Development of the cell immobilization technique as an important biosensor enabling technology, and integration of the immobilized cells in the biosensor system is described. The biosensor was used to test several classes of biologically active agents (environmental and bacterial toxins) categorized in the following classes: 1) neurotransmitters; 2) adenyl cyclase activators;3) cytoskeleton effectors; 4) cell membrane effectors; and 5) protein synthesis inhibitors. The obtained responses were analyzed and quantified by measuring the change in cell area covered by toxin, by using image analysis. Particular response features such as mode of response, its magnitude; kinetics, sensitivity and dose dependence were monitored and may be used as a basis for mathematical modeling of the responses. Streptococcus pyogenes streptolysin S and streptolysin O, Clostridium tetani tetanolysin, Staphylococcus aureus alpha-toxin, and Vibrio parahemolyticus hemolysin, all bacterial toxins which act on cell membrane, elicited a strong response from chromatophores. The results indicated that the biosensor has a great potential for the use in food and water testing and in pharmacy.

Keywords: fish chromatophores, biosensor, environmental toxins, bacterial toxins, biologically active agents

Lecture

Alternatives in pharmaceutical toxicology: global and focussed approaches

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Testing for the safety of a potential drug has been and maintains to be a challenging task for the toxicologists in the pharmaceutical industry. The emerging "new technologies" such as "omics" have increased the possibilities but also the expectations to assess relevant and species-specific toxicities of drug candidates. Nevertheless, the rapid pace of technologic development has not accelerated safety testing to the expected extent yet. In this review, I will give two examples for the application of target-specific cell models to detect and assess species-specific toxicities. Here, global and focussed analyses of gene-, protein and cellular changes have been successfully employed for the characterization of adrenal and hepatic toxicities. In the first example, we have used adrenal cell models based on primary as well as a permanent human adrenal cell line. Both cell systems enabled a good prediction of adrenal effects in rodents, non-rodents as well as humans. The second example made use of primary hepatocytes. In this project, a drug development candidate showed unexpected toxicities as well as species-specific cytochrome P450 induction (CYP) *in vivo*. We therefore analyzed CYP induction and potential toxicity signatures in rat and human hepatocytes as well as in samples from *in vivo* toxicity studies. By this approach, the rat hepatocyte model predicted correctly the effects observed in rodents. By comparing effects observed *in vitro* and *in vivo*, a solid extrapolation for effects in humans was possible. These examples show that an intelligent testing strategy using alternative methods can permit a meaningful safety assessment for humans if a "tailor-made" range of technologies along with "classical" toxicology is employed.

Keywords: expression profiling, in vitro models, primary cells, target-specific tests, mechanistic toxicology, explanatory toxicology

Poster

[Cytotoxical investigations with an electrochemical *in vitro* measuring method]

Zytotoxikologische Untersuchungen mit einem elektrochemischen *in vitro* Messverfahren

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Bei gesetzlich vorgeschriebenen toxikologischen Untersuchungen in Verbindung mit dem Inverkehrbringen von Pharmawirkstoffen, der Herstellung von Medizingütern und der Abschätzung von Chemikaliengefahren wird weltweit eine große Anzahl von verschiedenen Tests durchgeführt, um Aussagen über eine eventuelle schädliche Wirkung auf den menschlichen Organismus machen zu können. Zu diesem Zweck kommen in jüngster Zeit vermehrt photometrisch basierende Zytotoxizitätstets zum Einsatz. In dieser Arbeit wurde eine vorhandene Methodik der elektrochemischen Bioaktivitätssensorik als *in vitro* Screeningmethode zur Bestimmung der Zytotoxizität eingesetzt. Zur Anwendung kam dabei ein amperometrisches Messsystem (Dreielektrodensystem). Bei einem bestimmten Potential wurden so die reduzierenden Eigenschaften stoffwechselaktiver Organismen unter Einsatz von Redox-Mediatoren elektrochemisch erfasst. In Abhängigkeit der Menge von durch Organismen/Zellen reduzierten Mediatormolekülen entsteht ein Stromsignal. Das Stromsignal gibt je nach Ausgestaltung der Versuchsbedingungen entweder Aufschluss über die Anzahl von lebenden Organismen/Zellen oder bei vorgegebener Menge an Organismen/Zellen Aussagen über deren Stoffwechselzustand bzw. Stoffwechselaktivität. Bei entsprechender Zugabe einer Testsubstanz lässt sich hierbei in Abhängigkeit von der jeweiligen Zelltoxizität dieser Substanz auf das eingesetzte Zellsystem eine Verminderung des Stromsignals im Vergleich mit einem entsprechenden Blindwert ablesen. Bei einer hinreichend genau abgestuften Konzentrationsreihe einer Testsubstanz kann so schnell eine Zytotoxizitätskurve aufgenommen werden. Die Besonderheit der

dabei zum Einsatz kommenden elektrochemischen Messzelle ist, dass während der Messung alle Substanzen und Zellen in einem Polysaccharidgel immobilisiert werden. Dies Gel begünstigt zum einen, dass die Zellen, Mediator und Testsubstanz gleichmäßig im Volumen der Messzelle verteilt bleiben, zum anderen wird durch das aufgequollene Gel das Eindringen von Luft in den Testansatz erschwert, was eine essenzielle Bedingung für eine stabile Messungen mit diesem Messsystem darstellt. Die Immobilisierung der Substanzen und Zellen durch das Gel macht es zudem möglich, dass pulvrige Mediatoren und Testsubstanzen mit lipophilem Charakter ohne einen entsprechenden Lösungsvermittler direkt für die Tests eingesetzt werden können. Mit dieser Messzelle wurden erste Tests an Modellsubstanzen durchgeführt, welche den vergleich mit etablierten Zytotoxizitätstests stand hielten. Das Projekt wird aus Mitteln des Bundesministerium für Wirtschaft und Technologie über die AiF gefördert.

Keywords: Zytotoxizitätstets, Zellen, Amperometrie, Immobilisierung, Bioaktivitätssensorik

Lecture

EpiFlow perfusion culture improves *in vitro* assessment of acute and chronic toxicity

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In contrast to conventional static cell cultures perfusion cultures provide steady state conditions for nutrients and metabolites, organotypic conditions, over prolonged periods of time. In addition toxins can be administered at constant rates and concentrations either continuously or repeatedly. Perfusion culture further allows time series analyses of toxic effects as the outflowing culture medium can be collated at high time resolution. The cytotoxicity exerted by several toxic compounds (CdCl2, diquat and cyclosporine A) on epithelial monolayer cultures was investigated in static and perfusion culture using the EpiFlowTM system and compared to each other. The endpoints assessed over the whole intoxication interval were the release of lactate dehydrogenase (LDH), adenylate kinase (AK) and the potential of cells to reduce resazurin. The results showed a greater sensitivity of epithelial cells towards continuous toxin administration in perfusion cultures as compared to single or repeated dose administration in conventional static cultures. These differences can be ascribed to the different delivery rates of toxins to the cellular site of action. The cellular accumulation of CdCl2, for example, was higher in cells kept under perfusion conditions than in cells kept under standard static culture conditions. Time series analyses of LDH release into the culture medium were performed for both culture methods and evaluated by a newly developed deterministic population balance model of cell death, which delivers insight to the dynamics of cell deathduring toxin exposure.

Keywords: perfusion culture, epithelial cells, cadmium chloride

Lecture

The Bhas42 Cell Transformation Assay as a predictor of carcinogenicty

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Carcinogenesis has been shown to be a multistep process, which involves sequential genetic alterations in a single target cell, which cause subtle alterations in growth control and culminate in cells that are able to form malignant tumours. Genetic changes can result from spontaneous or carcinogen-induced alterations in DNA. Non-genotoxic mechanisms, that are at least initially independent of direct DNA damage, can play a causal role in carcinogenesis. *In vitro* cell transformation tests using BALB/c3T3 or C3H10T1/2 cells can simulate the process of animal two-stage carcinogenesis. A limitation of these methods however is the required time (4-8 weeks) for the expression of focus formation as an indication of transformation. In order to improve the experimental conditions Sasaki et. al developed a cell line, Bhas42 cells,

which was established for BALB/c 3T3 cells transfected with v-Ha-ras oncogene. Transformed foci can be efficiently induced in a single culture of the cells b y treatment with initiating and promoting agents within a period of not more than 2 weeks. The Bhas-system was established by using the standard reference carcinogen, 3-methylcholantrene. A reproducible dose-dependent increase in transformed foci (type III) was obtained with concentrations ranging from 0.05 to 3.0 µg/ml 3-methylcholanthrene. Also some of the type III foci were isolated by a trypsination technique. Growth characteristics of the type III cells were compared to that of the normal BALB/c 3T3 cells. FACS-analysis indicate a greater proportion of S-phase cells from the transformed phenotype compared to that of the parent cells. This indicates a distinctly higher proliferation rate of the transformed cells compared to normal cells. The Bhas-system could be easily established in our laboratory with the reference carcinogene 3-methylcholanthrene and the obtained results were reproducible and stable. Based on the different characteristics of transformed and non-transformed cells we are now committed in seeking molecular markers of morphological transformation which will lead to a more objective scoring of the different types of foci (type I, II and III) and a greater acceptance of the transformation systems.

Keywords: cell transformation, in vitro carcinogenicity, Bhas42 cells, alternative method

Poster

An *in vitro* model to test cosmetics and drugs on human hair follicles

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Normal human occipital scalp samples are obtained, from healthy male patients, during routine excision of benign scalp lesions (e.g. , naevi and cysts). Isolation of anagen hair follicles is achieved by using a surgical blade, microscissors and watchmakers forceps under a stereo dissecting microscope. As in micrograft preparation for hair transplantation procedures, each follicle is isolated intact as a whole, always maintaining a significant amount of tissue (epidermis, dermis, subcutaneous fat) around the entire length of the follicle. Alternative techniques for the recovery of follicles (as by digesting the skin with collagenase) have failed to yield follicles capable of supporting hair growth, being the maintenance of the structural integrity of the follicle (and related structures) an essential requirement for growth *in vitro*. Any follicle visibly damaged during dissection is discarded. The obtained follicles are divided are randomly assigned to a control Group or a n experimental Group. Follicles from both Groups are stored in 500 µl of Williams E medium with supplements as follows: 1% fetal calf serum, 10µg/ml transferring, 10 µg/ml insulin, 10 ng/ml sodium selenite, 10 ng/ml hydrocortisone, 100 U/ml penicillin, 100 µg/ml streotomycin, 2.5 µg/ml fungizone, and cultured for ten days. Supplemented medium is prepared fresh prior to experiment, and changed every 72 hours. In the culture medium of the experimental Group is added the substance to be tested. Follicles are maintained free floating in individual wells of 24 well multiwell plates in an atmosphere of 37°C, 5% CO^2/95% air, and 100% humidity. This allows detailed measurements to be made on the length of individual hair follicles. The length of each follicle is measured at magnification x20 immediately following the 5-hour test period and at the end of the 10-day culture period, using a microscope with a calibrated eye-piece graticule. Total follicle length is computed as the distance from the base of the bulb to the end of the shaft. Follicles which loose normal follicular architecture due to degeneration late in the culture period are computed as not survived. Histology is accomplished, at the end of the ten day culture period, by fixing the follicles in phosphate buffer saline (pH 7.4) containing 10% paraformaldehyde, embedding in paraffin wax, sectioning at 10µm thickness, and staining with the Heidenhein's "Azan trichromic" modified protocol: this procedure results in a red staining of the nuclei and of the internal root sheath, pink staining of the follicular papilla, dark blue staining of the matrix, violet staining of collagenous areas, orange staining of muscular tissues, and light yellow staining of the cortex of the hair, permitting a detailed analysis of the histology of cultured follicles. In our experience, the described method allows to collect some reliable data regarding the acute toxicity of peculiar cosmetics and drugs without the use of animal models.

Keywords: cosmetics, drugs, hair follicles

Poster

Something in the air? Progress towards the development of an analytical methodology for the detection and identification of immunomodulative airborne pollutants

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Numerous studies have reported the adverse effects of airborne pollutants on human health. However, only a small number have attempted to examine the immunomodulative potential of environmental pollutants and have tended to focus on the effects of single compounds. There appears to be a paucity of data concerning both the immunomodulative effects of complex environmental samples and the identity of causal agents. When investigations have been undertaken, these have often utilised animal models or sacrificial animal organ/cell donors. Our aim is therefore to develop an alternative *in vitro* methodology based on donated human cells to detect and identify airborne pollutants with immunomodulative potential in humans. Here, we outline our approach and report progress towards the development of our methodology. For initial validation, a number of common airborne pollutants were selected and assessed for their ability to modulate the proliferative response of human T and B cells to known mitogens. Isolated peripheral blood mononuclear cells were stimulated with either phytohemagglutinin or pansorbin and exposed to test compounds. Results were plotted to give the percentage increase or decrease in mitogenesis as a function of test compound concentration. Similar methods were then used to examine the immunomodulative potential of a number of samples of airborne particulate matter collected during and following an atmospheric temperature inversion in early 2006. Our results show that some common airborne pollutants including certain polycyclic aromatic hydrocarbons significantly inhibit the normal response of both human T and B cells to mitogenic stimulation. Extracts of airborne particulate material displayed highly significant inhibitive effects at concentrations equivalent to less than the amount of material present in one cubic metre of air. Samples collected during the temperature inversion displayed significantly higher immunomodulative potential than those collected during normal atmospheric conditions. Future work will involve the application of bioassay-directed chemical analysis and investigations into mechanisms of action of the causal agents.

Keywords: immunomodulative, airborne, pollutants

Poster

Baby-cream blues: An *in vitro* investigation into the estrogenic potential of infant skin-care products

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Recent research has shown a number of chemicals commonly added to so-called 'personal care products' (PCPs) such as sunscreens, shampoos and deodorants display significant estrogenic potential. Infant skin-care products (ISCPs or 'baby-creams') can be defined as PCPs applied to infant skin mainly for cosmetic reasons. In common with other PCPs, many ISCP formulations contain compounds that have been individually shown to display estrogenic potential *in vitro*. The often routine and extensive application of these potentially estrogenic compounds to immature human skin may therefore represent an important route of exposure. Our aim was to develop a suitable analytical methodology and apply this to commercially available ISCPs to gain an insight into the estrogenic potential of this group of products. Thirteen different ISCPs were purchased off-the-shelf and extracted by sealing inside 50 cm sections of pre-cleaned low-density polyethylene tubing and dialysing in n-hexane. Interferants were removed from the extracts using size exclusion chromatography and the extracts were assessed for estrogenic potential using a yeast-based estrogen screen (YES). Ten of the tested samples displayed significant concentration-response activity whereas no activity was observed in any process controls. Most sample curves were biphasic with higher concentrations of sample eliciting cytotoxic effects on

the yeast. 17b-estradiol equivalents (E2-EQ) were calculated for each of the active samples. These ranged from 5.5 to 170 ng/ml suggesting a high estrogenic potential associated with some ISCP formulations. Several of the most active samples were subsequently subjected to reverse phase chromatographic fractionation (HPLC) and re-tested using the YES. Charts of estrogenic activity as a function of retention time showed activity to be concentrated in the early eluting fractions. Initial analysis suggests that the majority of observed estrogenic activity is associated with para-substituted hydroxybenzoate esters (parabens) added to many PCPs as preservatives. Future work will involve assessing the estrogenic potency of the ISCP formulations using an estrogen responsive human cell line (MCF-7) and analytical confirmation of the active compounds.

Keywords: estrogenic, baby-creams, YES

Poster

Comparison of three methods in the sensitivity assessment of the *in vitro* micronucl eus test

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Many chemicals interact with genetic material, leading to chromosome aberrations. Such substances are classified as aneugenic or clastogenic. Different tests allow the characterisation and the classification of these chemicals. Among them, the chromosome aberration test and the micronucleus test permit detection of chromosome aberrations. Due to metaphase analysis, the first test presents some disadvantages. The second test is easier, the preparations can be scored more quickly and be assessed more objectively. Despite the fact that this type of assay is well-accepted and validated as an *in vivo* test (OECD Guideline 474), the *in vitro* method is not reflected in officially accepted test guidelines at OECD, just a draft is published to date (OECD draft proposal for a new guideline 487) and many disparities in the conductance of this test have become obvious. To contribute to standardisation, three different methodological approaches of the micronucleus test *in vitro* with V79 cells and cytokinesis block were compared at BSL Bioservice GmbH : GIEMSA staining, Acridine Orange staining (fluorescence) and flow cytometry. V79 cells were treated with three known clastogens (Ethyl methanesulfonate, Mytomycin C and Cyclophosphamide) and one aneugen (Colcemid®). All three assays yielded positive response for all test substances. However, sensibility and specificity differences between the three methods were noted. Thus, all three methods are appropriate for micronucleus detection, but variations between each other exist, in terms of result interpretation.

Keywords: in vitro micronucleus genotoxicity V79 Flow Cytometry

Lecture

Use of a standardised and validated long-term human hepatocyte culture system for repetitive analyses of drugs: Repeated administrations of acetaminophen reduces albumin and urea secretion

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Human hepatocytes are the in vitro system of choice to study drug-induced processes in man. Here, we present HEPAC2: A standardised and validated culture system in which human hepatocytes are maintained in HHMM (Human Hepatocyte Maintenance Medium) with HGF (hepatocyte growth factor) and EGF (epidermal growth factor). Cellular viability and hepatocellular functions were monitored daily. Albumin and urea production remained on a relatively constant

level for up to 2-3 weeks. Based on this, a standard protocol was established that allows repeated exposure of hepatocytes to test substances for studying drug metabolism. We used acetaminophen (AAP) to assay the feasibility of this system. Hepatocytes were exposed to AAP (100-2815 mg/l) for 24 h. Subsequently, the culture medium was replaced by medium without AAP and the same exposure scenario was repeated in intervals of 4 days. High doses of AAP (2815 mg/l) diminished urea production by 15-30% and albumin secretion by 70-80%. This effect was reversible. After removal of AAP, secretion of urea and albumin returned to control levels. AAP hepatotoxicity is caused by its biotransformation to the reactive metabolite N-acetyl-p-benzoquinone imine that is mediated by CYP2E1 and CYP1A2. Obviously, the AAP activating enzymes were active for at least 21 days and the activity was maintained during at least four repeated cycles of exposure to AAP. In conclusion, these data demonstrate the suitability of our long-term culture system to serve as a tool for repetitive screening of drug-mediated changes on hepatocellular functions. Thereby, this culture technique may help to overcome the sparse availability of human hepatocytes for testing drug-mediated responses in man.

Keywords: human hepatocytes, acetaminophen, toxicity, hepatocellular metabolism

Lecture

Finalizing REACH – an evaluation of the European Parliament's and the Council's position from the point of view of the German Animal Welfare Federation

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Based upon the framework depicted in the White Paper "Strategy for a future EU chemicals policy" published in February 2001, in October 2003 the European Commission put forward the draft for the new REACH regulation to the Council and the European Parliament for their decision. The aims of the so-called REACH system (Registration, Evaluation and Authorisation of Chemicals) depicted therein are better to protect humans and the environment from unwanted effects of chemical substances while at the same time taking into account economic considerations. In acknowledgement of the importance of these aims, the German Animal Welfare Federation submitted detailed comments on how they can be met without performing new animal tests. From the point of view of animal welfare, animal testing for the safety evaluation of chemical substances can be avoided, if the draft REACH regulation, amongst other issues, is amended to better ensure that all existing information related to hazardous effe cts of chemicals is made available and shared, that all available means to collect information without animal testing are made full use of and that only such information is collected that is required to ensure the safe use of a specific substance. After long and extensive debates, on 17 November 2005 the European Parliament voted on its amendments to the draft REACH regulation, and the Council adopted its opinion on 13 December 2005. Unfortunately, the changes decided upon by the Council do not reveal a clear tendency to improve animal welfare issues, and also the amendments adopted by the Parliament do not go far enough to address all concerns of animal welfare. For instance, while both sides did adopt provisions to strengthen the rules on data sharing, neither set of rules go far enough to make data sharing mandatory without exception. And while the provisions to ensure that only such data will be collected that are necessary for the safe handling of a given substance were improved, both the Council and the European Parliament failed to adapt the testing strategies to the state-of-the-art concerning available means to determine chemical hazard without animal testing. In the presentation an overview will be given over the animal welfare relevant amendments adopted by Council and Parliament as well as the common position currently in preparation, and these relevant documents will be commented on from the point of view of animal welfare.

Keywords: REACH, data sharing, flexible step-by-step non-animal testing strategy, regulatory toxicity testing, 3R, risk assessment

Poster

The use of transgenic animals in biomedical research in Germany – status report 2001-2003, ethical evaluation and examination of indispensability

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A total of 577 scientific articles on research projects with transgenic animals performed in Germany published in the years 2001 -2003 were collected with the aim of obtaining an overview over the goals and the contents of such fundamental research. According to the topics covered by the publications, main areas of biomedical research with transgenic animals can be found in the fields of neurobiology, immunology, cardiology, embryology and oncology. However their use can be discerned in all other areas of fundamental biomedical research as well. The vast majority of transgenic animals used were mice, followed by rats and pigs. Additionally, singular research projects with fish, rabbits and chicken were recorded. A high percentage of the rats were used in cardiovascular research, whereas transgenic pigs were produced and bred as organ donors in xenotransplantation research. As a rule, transgenic animals are being used in *in vivo* experiments to examine gene functions, their regulation or the contribution of genetic alterations to the development of diseases. Many transgenic animals already are affected in their wellbeing due to the genetic modification alone regardless of the procedures performed with them. An ethical evaluation showed that it is to be questioned whether the experimental use of transgenic animals led to results that were of such outstanding scientific relevance that they legitimated the suffering of the animals. In order to point to possible approaches to avoiding the use of transgenic animals in the areas of research identified, subsequent investigations aimed at collecting information on non-animal test methods that might be applied in pursuing the aforesaid questions. In particular, these were non-animal test methods that make use of genetic techniques. Amongst these are *in vitro* cell culture methods with genetically modified cells, such as the so called Transfected Cell Array, as well as *in vitro* test methods, in which specifically targeted genes can be turned on or off selectively for example with help of the so-called RNA interference technique. Since such technologies can also be applied to cell cultures with human cells, investigations with these methods enable direct information on the function of human genes. From the point of view of animal welfare the broad spectrum of already available non animal test methods with which to study the function of genes and genetically caused pathophysiological reactions shows that waiving of animal tests with transgenic animals is possible without impeding biomedical research as such.

Keywords: transgenic animals, genetic engineering, literature survey, cost-benefit-analysis, ethical evaluation, 3Rs, animal experimentation, gene technological non-animal test methods

Lecture

Regulatory animal testing

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Thirty percent of the animal tests conducted annually in Europe are performed to meet regulatory requirements pertaining to the authorization and release of a substance or product onto the European market. Regulatory animal testing is often repetitive in nature and more likely to cause severe suffering than other types of animal testing due to the procedures used. Because of these characteristics, regulatory animal testing is very interesting in terms of the 3R policy (Replace, Reduce and Refine). A survey (2005) was conducted into the actors and factors that influence the use of animals in testing to comply with regulatory requirements. Wherever possible, the research focussed on animal testing required by

protocol for the authorization and release of pharmaceuticals. Regulatory animal testing is a persistent element in the assessment procedures for registering a substance or product for release onto the market. Even though the number of alternative test methods keeps increasing, these new methods are not automatically included in assessment procedures. In order to increase the use of alternative methods to comply with regulatory requirements, a number of obstacles must first be overcome. Technical factors: In this field the greatest gain is expected from so-called strategic test approaches, data sharing and retrospectively analysing existing data. Political / administrative and social factors: Harmonization of legislation and regulations is a precondition for reducing regulatory animal testing. This survey identifies and describes the various opportunities and threats for the implementation of the 3Rs in regulatory animal testing. Further recommendations are: * use risk communication in order to influence the level of risk acceptance, * make the costs of conducting animal tests transparent, * widely publicize available alternatives, * improve communication between stakeholders, * strengthen the policy network.

Keywords: regulatory requirements, animals, testing, technical factors, political/administrative factors, social factors, harmonization

Poster

Usage of modified HET-CAM-assays to determine several phamacological activities of essential oils

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Nowadays many people prefer drugs of natural sources instead using synthetics. But often the knowledge of their pharmacological activities and safety is limited or based on historical experiences. So we decided to check different supposed pharmacological activities of several essential oils and their single components with a modern scientific method. A useful model was supposed to be the HET-CAM-Assay (Hen's Egg Test – Chorioallantoic Membrane) utilizing the CAM's capillary system of breeded hen eggs. We used different modifications of the HET-CAM-Assay to screen for the antiangiogenic, the antiinflammatory effect and also the irritant side effect of essential oils and their single components. Irritant side effect identification was done by determining the irritation threshold concentrations which is the first concentration causing bleeding of CAM´s vessels. Antiinflammatory activity was checked by ranking the phenomenons "star like vascularisation" and "granuloma formation". In addition a cyclooxygenase (COX) assay was done, an enzyme involved in inflammation process. Antiangiogenic activity was identified by detecting vessel free areas on the CAM. Ranking essential oils and their single components by their irritation threshold was very successful. All essential oils or components despite of sesquiterpenes caused haemorrhage on the CAM at least in case of undiluted application. Identifying an antiinflammatory effect of essential oils or their components using the HET-CAM-Assay failed. Is was not possible to create reproducible data not even for established antiinflammatory drugs like hydrocortison. Remarkalbe is that it was possible to show that essential oils do have inhibition potential on COX, and so on inflammation processes, especially oils containing phenylpropanes. Some essential oils and single components were also able to create vessel free areas on the CAM means to inhibit physiological angiogenesis. In conclusion the HET-CAM-Assay is a very useful detection model to determine the irritating and the antiangiogenic effect of essential oils. We also postulate that the HET-CAM-Assay is not able to give any antiinflammatory response because of the mature immune system of the chicken embryo.

Keywords: HET-CAM; essential oils; irritation; angiogenesis

Lecture

Hazard identification in chemical food safety: use of modeling, primary hepatocyte cultures and microarrays to address the level of concern for potential process contaminants of unknown toxicity

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The discovery in 2002 of acrylamide formation in heated foods triggered a still ongoing worldwide research effort to address human health significance. Although formation pathways, food levels and human exposure are currently largely established, no exposure- or food limits have been established to date. We had previously initiated a study that was based on the assumption that dependent on precursors present in food (free amino acids and reducing sugars), processes that trigger the formation of acrylamide (Maillard reaction) may similarly trigger the formation of other compounds that might raise concern. Modeling of formation, food levels, exposure and toxicity had led to the identification of several compounds that were prioritized by their potential level of concern based on margin of exposure (MOE). Using this approach, acrylamide turned out as the compound with the highest level of concern, whereas all other compounds significantly ranked behind. In order to confirm the results of the modeling study, 2 compounds that revealed a relatively low MOE and for which toxicological information was lacking, were selected for testing in an *in vitro* study using primary hepatocytes: 2- and 3-butenamide were studied in comparison to acrylamide. We used biochemical endpoints (cytotoxicity and glutathion depletion) as quantitative cytotoxicity parameters and Affymetrix microarrays to address qualitative aspects of the response to the compounds. Results will be presented that demonstrate that, in spite of very similar chemical structure compared to acrylamide, 2- and 3-butenamide are of much less cytotoxic potency. In addition, the two compounds induce changes in the gene expression profile that significantly differ from the response induced by acrylamide. We conclude that based on modeling and *in vitro* studies, potential processing contaminants can be ranked according to safety concern. Such an approach may allow to set priorities for further research.

Keywords: process contaminants, modeling, acrylamide, primary hepatocytes, microarrays

Lecture

Stem cell-based *in vitro* models as a basis to test efficacies of preclinical phases of anti-neurodegenerative treatments

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Next to toxicity tests, efficacy is the major issue before compounds can be transferred to clinical phases. Related animal models for some of the most important areas of human disease, e.g. cancer and neurodegeneration, require very laborious, sophisticated but often extremely disruptive, irksome and even cruel procedures. Functional, molecular and behavioural read-outs are an absolute necessity for pre-clinical therapeutic trials. Among examples there are xenograft models for various human cancers; models for stroke which induce severe cerebral ischemia by permanent occlusion of cerebral arteries in test animals (MCAO); various transgenic rodent models for neurodegenerative diseases, or extremely tough experimental models for autoimmune encephalomyelitis induced by vaccination of test animals with autoantigens as preclinical models of multiple sclerosis (MOG-EAE). Despite a high degree of sophistication, there is a general sense of caution towards these models, because often their read-outs are insufficient or misleading. Taken together, in the area of efficacy testing, there is an urgent need for the development of novel *in vitro* methods, which can balance disadvantages in terms of organ specific barriers and metabolism with advantages regarding the higher relevance of humanised systems, more precise functional and molecular read-outs and potential of higher throughput. In the given situation

of regulatory frameworks in the EC and elsewhere, again the purely diagnostic use of human embryonic stem cells models appears to develop into a highly attractive alternative. They can be differentiated to organotypic cell cultures and serve as flexible substrate for a variety of functional molecular endpoints. Moreover they are ideal for validation purposes using modern silencing technologies in a framework of genetically homogeneous differentiations to various "tissue"-like cell culture substrates like neural cells, cardiomyocytes, various types of muscle cells and adipocytes, etc. Here we show results from initial phases of a project which attempts to explore the outstanding potential of corresponding human embryonic stem cell (hESC)-based *in vitro* models. Related results from corresponding murine ESC-screening systems, in combination with quantitative differential proteomic display techniques, have already provided biomarkers for potential replacement of some related animal models.

Keywords: embryonic stem cells, MCAO, MOG-EAE, neuroprotection, neurodegeneration

Poster

Characterisation of morphology and barrier properties of a new human corneal epithelial model

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Institute of Pharmacy, Free University of Berlin, G-Berlin The corneal epithelial multilayer represents the principal barrier in the ocular pathway and is involved in corneal inflammation and wound healing processes. An immortalised human corneal cell (HCE) line (Araki-Sasaki, 1995) has been used to reconstruct the corneal epithelium. This model has proved to be a useful tool for both drug toxicity and permeation studies. It was the aim of this study to optimise the morphology and barrier properties of the epithelial layer with respect to different culture conditions. HCE were cultivated in cell culture inserts. The effect of mediums containing different serum and calcium concentrations were followed for 16 days. Growing rate, number of cell layers and epithelial differentiation were evaluated by cryosectioning and light microscopy. Barrier properties were determined by transepithelial electrical resistance (TEER) together with immunohistochemistry of the tight junction related protein claudin-1. Epitheliums cultivated in high serum concentration showed hyper-proliferation (about 13 cell layers at day 8 and 23 cell layers at day 16). In contrast, serum-free culture conditions using mediums supplemented with calcium resulted in a morphology which is closer to *in vivo* conditions (5 layers at day 8 and 7 layers at day 16). According to our TEER measurements and claudin-1 expression, high serum concentration reduced the barrier properties compared to serum-free conditions, whereas calcium seemed to increase them. In conclusion, serum free culture conditions paralleled by high calcium concentrations are the most promising conditions for adequate number of cell layers and effective barrier function of reconstructed epithelia.

Keywords: human corneal epithelial model, immortalised epithelial cells, transepithelial electrical resis tance, immunohistochemistry

Lecture

10-day medium throughput *in vitro* screen to assess chronic cytotoxicity of nucleoside analogues

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Sandwich Nucleoside analogues (NUC) are a major class of antiviral drugs that target viral DNA polymerases. They are well established in the treatment of HIV infection and are also under evaluation as newer treatment strategies against the hepatitis B and C viruses. Long-term treatment with NUC is however, associated with adverse side effects often attributed to inhibition of mitochondrial DNA (mtDNA) synthesis that results in gradual impairment of mitochondrial

function. As clinical toxicity normally develops only after long-term NUC therapy, *in vitro* investigations tend to involve chronic modelling for periods of up to 30 days. We required a suitable and efficient *in vitro* toxicity screen for NUC that could be applied at the drug discovery stage as part of a strategy to reduce the rate of late-stage attrition of new drug candidates. Accordingly we designed a chronic cytotoxicity assessment model (CCAM) with HepG2 human hepatoma cells grown in 96-well microplates without the nee d to subculture, and which allows co-measurement of cell count/viability and mtDNA. The format includes generation of nine-point dose-response curves using triplicate wells per drug concentration with treatment replenishment three times per week. After 4, 7 and 10-days' treatment, cells are assessed for growth and viability with nuclear dyes (Guava ViaCount)and counted on a Guava PCA-96 flow cytometer after which mtDNA (day 4) is measured by PCR. Degree of toxicity is determined by the rate of decrease in therapeutic index (IC_{50}:Cmax or IC_{50}:antiviral IC_{50} ratios) with time and/or whether a threshold TI is exceeded. Dependence of toxicity on mtDNA depletion is determined by relative displacement of dose-response curves for cell count and mtDNA levels. Five anti-AIDS NUC were used to validate the model, for which there was close concordance between degree of *in vitro* toxicity and severity of clinical toxicity. After 4 days' treatment, the relative displacement of mtDNA do se-response curves from those for cell count accurately predicted the differential dependence of toxicity of NUC on mtDNA depletion. CCAM offers considerable savings over animal studies, only requires a total of 10-15 mg compound, and is conducive to the 3Rs principle. We now use CCAM for routine screening.

Keywords: chronic cytotoxicity screen, nucleoside analogues, HepG2 cells, cell growth, mitochondria, mtDNA

Lecture

An *in vitro* model for early embryonic development

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Embryonic stem cells give rise to embryoid bodies which undergo developmental processes at the molecular, cellular and morphological level reminiscent of eutherian embryonic development [1]. Defined culture conditions and epigenetic status of embryonic stem cells allow to mimic pregastrulation development and early gastrulation *in vitro*. We could demonstrate that extra-embryonic endoderm forms properly and influences early gastrulation giving rise to all three germ layers [2]. Specific modulation of primitive mesoderm by extra-embryonic endoderm derived growth factors allows reproducible development of large numbers of cardiomyocytes [3-5], which so far continues to proliferate for 35 days and to contract rhythmically for 150 days. This model allows to study early developmental processes *in vitro* in basic science avoiding some experiments on living embryos, and provide a source of cardiomyocytes which may be used for applied long term toxicological studies. Further development of this model beyond gastrulation into organogenesis, however, challenges the question whether this ends justify the means?

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Keywords: embryonic stem cells, embryoid bodies, embryogenesis, gastrulation, cardiomyogenesis, growth factors

Lecture

Application of DNA microarrays for immunotoxicological testing

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The goal of our present research is the development of a DNA microarray as an *in vitro* toxicological test system to reveal the sensitising potential of chemicals. Dendritic cells from human donors are used to analyse gene expression after treatment with different potential allergens. Furthermore, the microarray shall be used to discriminate between contact

allergens and respiratory allergens. We developed a DNA microarray containing 65 immune genes, housekeeping genes, negative controls and external spike controls for normalization. Different labelling techniques were tested in order to optimize the system, especially taking into account the limited amount of immune cells and RNA available for the microarray assay. For protocol optimization the human monocytic cell line THP-1 was used, which can be cultivated easily and leads to higher RNA amounts compared to immune cells. The cells were stimulated with LPS and gene expression in LPS-treated cells was compared to gene expression in untreated cells. Applying a direct labelling protocol, increased expression of several immune relevant genes could be shown with either 10 µg or only 2 µg of total RNA. However, more upregulated genes could be detected with an indirect labelling method. Again, specific signals could already be obtained after using only 2 µg of total RNA. Based on these results, we now use the indirect labelling protocol for the microarray analysis. In order to find candidate genes for the identification of immunotoxic chemicals, nickel sulfate and other model allergens are applied to dendritic cells and gene expression is compared to gene expression in untreated cells. For the analysis of yet uncharacterized chemicals, a general treatment procedure has to be established and a time point for expression analysis has to be defined. Therefore, expression patterns at different time points after application of known allergens are compared. Our preliminary results indicate that the microarray technology could provide an *in vitro* alternative to animal test methods currently available to predict the sensitizing effects of chemicals.

Keywords: DNA microarrays, dendritic cells, immunotoxicological testing

Poster

Examination of ocular irritancy by the HET-CAM test

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Agrochemicals must undergo numerous toxicological tests before registration. Using animals in toxicological screening is a controversial issue. The Draize eye irritation test is one of the most criticized methods because of the injuries inflicted on the test animals. Several *in vitro* methods have been used to investigate the toxicity of potential eye irritants with a view to replacing *in vivo* eye irritation testing. In the HET-CAM test chemicals are placed in direct contact with chorioallantoic membrane of the hen's egg. The occurrence of vascular injury or coagulation in response to a compound is the basis for employing this technique as an indication of the likelihood that a chemical would damage mucous membranes (especially the eye) *in vivo*. The CAM is a complete tissue including arteries, capillaries and veins, and is technically easy to study. It responds to injury with a complete inflammatory reaction, similar to the tissue of the rabbit eye. In our studies comparative screening was performed with a set of agrochemicals to establish paralell data on *in vitro* (HET-CAM) and *in vivo* (Draize) results. The test materials were: Totril (ioxynil), Omite 57 E (propargite), Actellic 50 EC (pirimiphos methyl), Stomp 330 EC (pendimethalin), Mospilan 3 EC (acetamiprid), Alirox 80 EC (EPTC), Thionex 35 EC (endosulfan), Pyrinex 48 EC (chlorpyrifos). Agrochemicals to be tested are added to the membrane and left in contact for 5 minutes and the membrane is examined for vascular damage at set time periods. Irritancy is scored according to the severity and speed at which damage occurs providing an indication of the likely irritant effect of the compound. Our study showed good correlation between results obtained by the HET-CAM test and those of the Draize rabbit eye test most cases. The present form of the HET-CAM test can be proposed as a prescreen method of eye irritation tests, therefore the number of test animals can be reduced.

Keywords: agrochemicals, pesticides, in vitro, ocular irritation, chorioallantoic membrane

Lecture

Cell culture model for colon carcinogenesis, chemoprevention and organ-selective toxicity

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Germ line mutation in the tumor suppressor adenomatous polyposis coli (APC) gene represents a primary genetic defect in the clinical familial adenomatous polyposis (FAP) syndrome, predisposing for colon cancer. Targeted chain termination mutation on codon 1638 of the mouse APC gene promotes small intestinal carcinogenesis predominantly in the small intestine. Administration of a chemical carcinogen or a tumor promoting high fat/ low micronutrient diet to APC mutant mice, however, induces colon carcinogenesis. A reliable cell culture model established from appropriate target organ that expresses relevant genetic defect and quantifiable risk for carcinogenesis should provide an innovative complementary approach to long term animal studies on colon carcinogenicity, chemoprevention and organ selective toxicity. The objective of this study was to develop a reliable preclinical cell culture model for human FAP syndrome and validate this model as a rapid mechanism-based approach for screening efficacious preventive/therapeutic compounds.

The newly developed epithelial cell line 1638N COL from morphologically normal colon of haplo-insufficient APC 1638N +/- mutant mouse exhibited aberrant cell cycle progression, down-regulated apoptosis, enhanced risk for carcinogenesis *in vitro* and tumorigenesis *in vivo*. The aberrantly proliferative tumorigenic APC mutant cells also exhibited about a 2-4 fold higher constitutive expression of β-catenin, cyclin D1 and COX-2 proteins, relative to that in the wild type colon epithelial cells expressing the APC +/+ genotype. Treatment of APC mutant cells with low dose, non-toxic combinations of mechanistically distinct chemopreventive/ therapeutic agents resulted in about a 3 fold higher efficacy for cytostatic growth arrest, and about a 20-35% higher efficacy for inhibition of the risk for carcinogenesis, relative to that exhibited by these agents used independently.

These data establish a novel cell culture model for clinical FAP syndrome and validate a rapid, mechanism-based screening approach to prioritize efficacious combinations of preventive/ therapeutic agents for long term *in vivo* animal studies and future clinical trials on colon cancer prevention. [Support: The Irving Weinstein Foundation and NCI MAO # CN-75029-63].

Keywords: epithelial cell culture, gene mutation, cancer prevention

Poster

Innovative testing strategies as alternatives to animal testing, an international course

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With the intensifying demand on alternatives to animal experiments in industry and to address fundamental scientific questions, the need for qualified technical and scientific personnel is steadily growing. It will become increasingly important to educate technicians specialized to develop and perform alternatives models and have a critical view on experimental designs of animal studies for optimal use of animal data. In order to address this need a course on alternatives to animal use in life sciences research will start in September 2006. Students engaged in bachelor-level education in life sciences can participate in this course. The aim of the course is to acquaint students with both theoretical and practical aspects. The focus will be not only on development of single replacements for individual animal experiment, but to educate students in innovative testing strategies resulting in optimal benefit from animal and non-animal data. The up to date 6-month course consists of several thematically and interdisciplinary modules, including the three R's principle, Legislation, *in vitro* and *in silico* alternatives in Efficacy & Safety testing, Toxicological risk assessment in industry, Validation issues, Data management, bioinformatics and computer modelling, Ethics and Public opinion. One important feature of the course will be the project \'Mission Alternative\'. Throughout the course participants will work together with research institutes and industry to solve an existing problem or answer a scientific ques-

tion, regarding alternatives to animal use. Currently, we are attracting lecturers from abroad to enhance the international character of the course. To facilitate participation for international students this course will be developed in close collaboration with the international office of our university and foreign contacts. The course has been set up in close collaboration with the Dutch Organization for Applied Scientific Research, TNO. This resulted in an excellent program on both conceptual and practical aspects.

Keywords: education, alternatives

Poster

Ethical and legal aspects in animal experiments in Sri Lanka

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Ethics is a philosophical state of mind, required to make a correct judgment. Each country has a specific moral discipline related to their cultural and religious background. In the Sri Lankan context, being a majority Buddhist nation, animal welfare as well as animal rights issues take on added prominence. According to the paper titled Animal Welfare Legislation in Sri Lanka (proposal for law reforms on animal welfare legislation), of the 16 enactments that deal with animal and animal welfare, only four statutes have been passed during the post independence era (1948). A recent upsurge in interest in these issues has led to the development of a Draft Act awaiting parliamentary approval in Sri Lanka, which seeks to replace the antiquated Prevention of Cruelty to Animal Ordinance No. 13 of 1907 and also to implement several lapses in other legislations relating to animal welfare such as the statute to regulate the use of animals for scientific research. The Sri Lankan public is mostly unaware of animal experiments. However, it is the duty of the scientific community to carry out animal research where needed for the well being of the society; the society being comprised of both humans and animals. Thus there is a requirement for proper ethical control of animal experiments. If this aspect is not given adequate consideration, as soon as the public does become more aware of animal experimentation a huge controversy is bound to arise. As can be clearly seen in the above discussion, in addition to ethical controls to animal experimentation, alternative techniques are important to enhance our research efforts. We look forward eagerly to learn as much as possible about this relatively new and developing area of alternatives for animal experiments, as we in Sri Lanka feel it is especially relevant and appropriate to the local cultural situation.

Keywords: Sri Lanka, animal welfare

Lecture

Using of an *in vitro* system for prediction of hepatotoxic effects in primary human hepatoctyes

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Prediction of toxicity and compound responses in hepatocytes continues to be a major concern for the pharmaceutical industry. Therefore, *in vitro* studies of liver cells are important investigations for toxicity and drug compatibility and can help to reduce animal trials. Furthermore an important question is: How compatible are results of animal testing with human response? The advantage of *in vitro* systems is the usability of human cells. But immortalised human cell lines do not behave like primary cells. Non-immortalised primary cells may best represent normal physiology. In the present study primary human hepatocytes were used for an *in vitro* test in our Bionas® 2500 analysing system. This analysing system is able to monitor oxygen consumption, acidification and adhesion on six silicon chips in parallel. Modifications of these

parameters in response to test compounds reflect effects on the cell metabolism. All parameters are detected continuously and online during the long term measurement (up to several days). As an additional advantage regeneration and recovery effects can also be monitored respectively. In this study we have cultured primary human hepatocytes on collagen pre-coated chips in chemically defined Human Hepatocyte Maintenance Medium and, for comparison, in conventional two-dimensional cultures. The optimised medium allows cultivation of functionally differentiated hepatocytes for several weeks. In this study we compared the sensor chip based *in vitro* results with standard assays for hepatocytes like albumin release and urea release. We have investigated effects of acetaminophen (AAP) on primary hepatocytes. The hepatocytes were exposed to AAP (50-2815 mg/l) for 24 h. Primarily, cell respiration was obviously inhibited by AAP concentrations from 500 mg/l. Whereas cell adhesion was marginally reduced. In conventional cultures AAP administration had no effect on cellular viability. High doses of AAP (2815 mg/l) diminished urea pr oduction by 15-30% and albumin secretion by 70-80%.

Keywords: human primary hepatocytes, in vitro, online monitoring, cell metabolism

Lecture

Daphnia magna as alternative bioobject in ecotoxicology

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We have been developing non-traditional methods of the identification of pollutants, using various hydrobionts as biological objects and the study of the mechanism of toxic action of xenobiotics. The experiments were carried out with using of Daphnia magna. Daphnia magna is a Crustacean in the order of Cladocera. This aquatic animal extensively used as a test organism in aquatic toxicology due to their small size, short life cycle and amenability to lab culture. Daphnia magna is the most sensitive test-object in relation of different pollutants among all known biological objects including experimental animals. Experiments were performed with a 2-days old culture of Daphnia magna. The toxicity of xenobiotics was determined by the value of LC_{50}, a concentration of the compounds causing death to 50% of hydrobionts during incubation with toxicants for 24 hours. In the first stage of the work, toxicity of organophosphates (Dipterex, DFP, DDVP, Paraoxon, Malathion, Malaoxon), heavy metals ions (Hg, Pb, Cu, Co, Cd, Cr, As, Al), organochlorines (Aldrin, Dieldrin, Endrin, Aroclor, DDT, Lindane, PCBs etc.), cyanides (sodium cyanide) and pyrethroids (Cypermethrin, Fenvalerate, Deltamethrin, Permethrin, Allethrin, Resmethrin, Phenothrin, Kadethrin, Cyphenothrin) was determined. The effects of a number of antagonists on the toxicity of xenobiotics were studied. At the first time we discovered that in experiments to Daphnia magna some muscarinic cholinoreceptor blockers (atropine, amyzil etc.) reduced a toxic the effect of organophosphates. In the case of heavy metals the chelating agents (EDTA , Dithioethylcarbamate, Unithiolum, Sodium thiosulphuricum, L-Aspartic acid) were effective, for certain organochlorine poisonings - anticonvulsive drugs (diazepam, phenobarbital), for cyanide poisoning sodium nitrite and anticyane. In the case of pyrethroid's poisonings the antagonist of glutamate receptor (ketamine) and agonists of GABA-receptor (phenazepam, ethanol) reduced the toxicity of xenobiotics. As far as these antidotes have a specific treatment action only against definite classes of pollutants, we have elaborated the sensitive express-methods of bioidentification of pollutants.

Keywords: daphia magna, pesticides, bioidentification

Poster

The usage of Daphnia magna for screening of selective muscarinic cholinergic receptors antagonists

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In the experiments on the rats we have developed methodological approach to the evaluation of the selectivity of muscarinic cholinergic receptors (M-ChR) antagonists action in the whole organism conditions. According the results obtained during investigation the protective effect of M cholinolytics during acute poisonings of organophosphates (DDVP, DFP etc.) depends on M1 subtype ChR occupation. The efficiency of antagonists in inhibition of tremor reaction caused by M-ChR agonist arecoline administration associates with interaction of M2 subtype of ChR. It was established by the method of linear regression, that there was a high degree of correlation (r=0.99) for different M cholinolytics between the ratios of ED50 of M antagonists in the tests with arecoline and organophosphates and the ratios of dissociation constants of antagonists complexes with M-ChR from the homogenates of rat\'s cerebral cortex and heart containing M1 and M2 ChR subtypes respectively. Thus, the ratio of ED_{50} arecoline/ ED_{50} DDVP is serve as a measure of the selectivity of drugs action. Pharmacological analysis of Daphnia magna cholinergic system with the use of anticholinesterase compounds, and M-ChR agonists and antagonists was carried out. On the experiments to Daphnia magna the effects of some non selective, mainly M1 and M2 ChR antagonists on the toxicity of DDVP and arecoline were studied. There was a strong correlation between the ED_{50} of antagonists in the tests with arecoline and DDVP in the experiments on rats and the EC_{50} of antagonists in experiments on Daphnia magna. For the first time in the experiments on Daphnia magna it was shown that a ratio of the average effective concentrations (EC_{50}) of M antagonists in the tests with arecoline and organophosphates also may be used as a measure of the selectivity of M-ChR antagonists action. The principal similarity in action of muscarinic antagonists to Daphnia magna and rats allow to recommend the Daphnia for screening of selective muscarinic receptor antagonists.

Keywords: Daphnia magna, screening,cholinilytics

Lecture

Criteria for expert assessment by animal experiments committees

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Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes is currently being revised. The Technical Expert Working Sub-Group on ethical review proposes that part of the ethical review process should take place at the local level. Suggestions are also made with respect to the competencies of the participants in the ethical review process. In the Netherlands, ethical review is mandatory since 1997. The Dutch Act on Animal Experimentation (1996) requires that animal experiments committees (AECs) should review animal experiments and balance the scientific and societal interests of the experiments against the suffering of the experimental animals. According to the Dutch regulations the AECs have to be composed of at least seven members. The AECs should equally represent competencies on experimental animals, alternatives to laboratory animals, ethics and finally on animal welfare and protection. Criteria that have to be met in order to be regarded as expert in one or more of these areas have not been described. The competence of the AEC members can therefore not be guaranteed. Recently, the Dutch Act has been evaluated. The evaluation committee recommends that criteria have to be developed for the competence of members of AECs In this study, representatives of the four competencies were consulted in order to draft criteria which include both adequate knowledge and competence but that also allow a sufficient number of people to qualify for satisfying the composition of the AECs. Furthermore, it is claimed that in order to keep the knowledge of each expert up-to-date, compulsory continuing education of AEC members is required. Education should cover general items like statistics, alternatives, ethics, legislation, discussion techniques, and the importance of con-

sensus. In addition, compulsory courses are proposed to regularly update each competence on developments in their respective areas. The results of this study may serve as a starting point for further discussions on criteria for AECs members both in the Netherlands and in other countries.

Keywords: animal ethics committees, ethical review process, criteria, expertises

Poster

Relevance of the *in vitro* and *in vivo* studies in genotoxicological research on fibres

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Asbestos fibres can be considered as archetypes of toxic mineral fibres. Long-term exposure to the fibres can initiate asbestosis, bronchial carcinoma and pleural or peritoneal mesothelioma. Oxidative damage caused by the fibres may studied in *in vitro* genotoxicity tests involving analyses of bacterial mutagenicity or cytogenetic alterations in cultured cells. But the crucial endpoint (mesothelioma) is very difficult to study in an *in vitro* system. Mesothelial cell cultures show very short proliferative lifespan and early onset of senescence. Human peritoneal mesothelial cells were found only few (appr. 6) population doublings prior to senescence. Cells of the aging cultures generate more reactive oxygene species and display increased quantity of oxidatively modified DNA. Therefore they are not suitable for testing similar induced effects by fibres and long-term exposures. We developed an *in vivo* mesothelioma-induction test for research on fibrous materials. The test involves one single treatment and simple surgical methods by using minimal number of rats. Our model provides the opportunity of new classes of fibrous materials like tubes produced by nanotechnology. We concluded that only a battery of *in vitro* and *in vivo* studies can provide relevant data for the human risk assessment.

Keywords: fibres, asbestos, mesothelioma, genotoxicity

Lecture

The challenge of predicting drug toxicity *in silico*

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Poor pharmacokinetics and toxicity are not only frequent causes of late-stage failures in drug development but also a source for unnecessary animal tests. In drug discovery and for the assessment of the toxic potential of chemicals, *in silico* techniques are nowadays considered as valuable alternatives to *in vivo* approaches. Computer-based concepts allow to study both existing and hypothetical compounds; the methods are fast, reproducible, and typically based on human bioregulators, making the question of transferability obsolete. In the recent past, our laboratory contributed towards the development of *in silico* concepts (→ multi- dimensional QSAR) and validated a series of "virtual test kits" based on the estrogen, androgen, thyroid, and aryl hydrocarbon receptor (endocrine disruption, receptor-mediated toxicity) as well as on the enzyme cytochrome P450 (metabolic transformations, drug-drug interactions). The test kits are based on the three-dimensional structure of their target protein (i.e. $ER^{\alpha\beta}$, AR, $TR^{\alpha\beta}$, CYP450 3A4) or a surrogate thereof (AhR) and were trained using a representative selection of 362 substances. Subsequent evaluation of 107 compounds different therefrom showed that binding affinities are predicted close to experimental uncertainty. These results suggest that our approach is suited for the *in silico* identification of adverse effects triggered by drugs and chemicals and encouraged us to compile an Internet Database for the virtual screening of drugs and chemicals for toxic effects.

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Keywords: virtual test kits, in silico prediction of harmful effects triggered by drugs and chemicals, reducing animal testing

Poster

Virtual test kits for predicting chemical toxicity *in silico*

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The Biographics Laboratory 3R has developed and validated a series of virtual test kits to identify the toxic potential of both existing and hypothetical chemicals. Presently, our pilot simulation (cf. http://www.biograf.ch/GIFS/Tox DataBase.gif) focusses on endrocrine disruption (estrogen, androgen, thyroid and aryl hydrocarbon receptor) as well as on metabolic events mediated by cytochrome P450 (cf. http://www.biograf.ch/projects.html). The models were trained using a representative selection of 362 substances. Subsequent evaluation of 107 compounds different therefrom showed that binding affinities are predicted close to experimental uncertainty. These results suggest that our approach is suited for the *in silico* identification of adverse effects triggered by drugs and chemicals and encouraged us to compile an Internet Database for the virtual screening of drugs and chemicals for toxic effects.

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Keywords: ToxDataBase, in silico, reduction of animal models

Poster

Training course on the 3Rs principle

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Due to the misunderstanding of the term alternatives and the confusion of many people who think that it refers only to replacement, it is of great interest, the development of a special course on alternatives and the 3Rs principle, in order to clarify concepts. A training course in the 3Rs principle has been developed in the last years in the Faculty of Pharmacy of the University of Barcelona. This is a 30 hours post-graduated course based on theoretic and practical classes. The people attendant it, are principally, post-graduates in Pharmacy and Biology, but it is open to other graduates. The objectives of the course are to introduce students on the 3Rs principle and the use of alternatives to animals according to the EU policies on cosmetics and chemicals, the so-called REACH (Registration, Evaluation and Authorisation of Chemicals). After an introduction on the history of the 3Rs we focused the course in the most relevant alternative methods of toxicology *in vitro*. Especial attention is paid on the performance of some scientifically validated *in vitro* methods which are accepted by regulatory toxicology and the statistical evaluation of the test results. Among these test, the chorioallantoic membrane based test, the red blood cell haemolysis and the cytotoxicity in cell lines are evaluated. The search of alternatives and the information, on the more relevant webs, constitutes an important part of this course, providing the media to develop a search strategy using the more appropriate keywords and concepts. The students are introduced in the most relevant institutions involved in the development and

validation of alternatives. Special attention is paid on alternatives in education and their advantages in front of the traditional practices with animals. We provide to our students experience to apply the different methodologies to their own research and especially, to promote the use of alternative methods.

Keywords: 3Rs, alternatives, course, education

Poster

In vitro alternative to evaluate the potential antioxidant capacity of polyphenols from different sources

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The evaluation of the antioxidant capacity of new compounds is usually tested in animals. The *in vivo* study is performed in rats pre-treated by gavage with the product and the determination of the serum levels of the hepatic enzyme markers aspartate aminotransferase and alanine aminotransferase and the indicators of oxidative stress in the liver, such as the glutathine disulfide content and lipid peroxidation are measured. In other determinations rat liver microsomes are used. A possible alternative to the animal use, is the chemical determination, nevertheless, biological determinations have been demonstrated to be more useful. We have studied the potential antioxidant activity of polyphenolic fractions obtained from pine, hamammelis, and grape. The mixtures contain mainly flavanols (catechins) of similar degree of polymerisation and different percentage of pyrogallol groups in the following order: hammamelis > grape > pine. The *in vitro* oxidative haemolysis of human red blood cells (RBCs) was used as a model to study the free radical-induced damage of biological membranes and the protective effect of the flavanols. The haemolysis of RBCs was induced by a water-soluble free radical initiator 2,2\'-azobis(2-methylpropionamidine) dihydrochloride (AAPH). Whole blood obtained from healthy volunteers was analysed in this experiment. Erythrocytes were separated from plasma by centrifugation, washed and suspended in PBS solution. The addition of AAPH (a peroxyl radical initiator) to the RBC suspension causes the oxidation of lipids and proteins in cell membrane and thereby induces haemolysis. It is known that AAPH-induced haemolysis in RBC is a function of incubation time and is proportional to the concentration of free radicals. The inhibitory effect on RBC haemolysis is also proportional to the concentration of antioxidants in the incubation mixture. The incubation mixture was shaken gently in a water bath at 37°C for 2.5 h. Our study demonstrated that the biological test *in vitro* is an excellent model for the evaluation of antioxidant capacity of putatively biologically active compounds obtained from different natural sources, without the use of laboratory animals.

Keywords: antioxidants, red blood cells, haemolysis

Lecture

Estimation of the embryotoxic potency of valproic acid derivatives *in vitro* by using the embryonic stem cell test

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The Embryonic Stem Cell Test (EST) takes advantage of the potential of murine embryonic stem (ES) cells to differentiate in culture to test embryotoxicity *in vitro.* The test is based on the most important mechanisms in embryotoxicity – cytotoxicity and inhibition of differentiation - as well as on differences in sensitivity between differentiated and embryonic tissues. For the classification of compounds according to their embryotoxic potential we analyse the beating cardiomyocytes in embryoid body (EB) outgrowths and compare the results to cytotoxic effects on undifferentiated ES cells and differentiated 3T3 fibroblasts. Through a number of prevalidation and validation studies the EST was demonstrated to be a reliable alternative method for differentiating of test compounds between three classes of embryotoxicity: strongly, weakly and non embryotoxic. Recent improvements of the EST protocol using flow cytometry analysis showed that differential expression of cardiac specific proteins is a useful marker for detecting the embryotoxic potential of test compounds. The aim of the present study is to investigate whether the EST is sensitive enough to discriminate between structurally related compounds with gradually different embryotoxic potential. VPA is a potent antiepileptic drug with known teratogenic impact in humans and rodents. VPA derivatives, which were synthesised and highly purified at the University of Veterinary Medicine Hannover, exhibit different embryotoxic potentials, from *in vivo* highly to non teratogenic. Our results show that we are able to reflect structure-activity relationships of the VPA derivatives *in vitro* by using the validated and the new established molecular endpoints of the EST.

Keywords: Embryotoxicity, Embryonic Stem Cell Test, in vitro, Valproic acid

Lecture

Results of the validation study on percutaneous absorption via reconstructed human epidermis

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The REACH initiative of the European Community will increase the efforts for toxicological testing, hazard analysis and risk assessment. Since the skin is an important exposure route for chemicals, reconstructed human epidermis (RHE) models are of increasing interest for regulatory testing. Nine laboratories plus the ZEBET ran a validation study to qualify RHE for *in vitro* percutaneous absorption studies.Nine substances of varying physicochemical characteristics includ-

ing the OECD standard substances caffeine, testosterone and benzoic acid were applied to human epidermis sheets (HES), pig skin and RHE (EpiDerm™, EPISKIN™ and SkinEthic®) mounted into Franz cells. Permeation experiments were run in parallel in the partner laboratories based on a standardised protocol. Finite dose experiments were finally conducted. Although some variability was observed in the data, the results showed an acceptable inter- and intra-laboratory reproducibility. The permeation of test substances agreed well with the expectation, high molecular weight substances as well as hydrophilic agents showed low penetration. However, RHE was more permeable than pig skin and HES. Due to enormous scatter of benzoic acid permeation of HES and RHE, which were not based on analytical or stability problems, benzoic acid was judged to be unsuitable as a standard compound. Based on the results, it appears possible to develop a prediction model for correlating RHE to native human and pig skin.

Keywords: skin absorption; reconstructed human epidermis; alternative methods; human skin models

Poster

Acute toxicity of selected chemicals in adult zebrafish (*Danio rerio*) and its early life stages – the comparative study

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One of the methods for determination of acute toxicity of chemical substances is their assessment on fish. A possible way to accelerate the testing of chemicals on fish can be the use its early life stages (embryos in eggs). We compared acute toxicity data of eight different chemicals in adult zebrafish *Danio rerio* to fish eggs. Tests on zebrafish eggs were performed according to the OECD Guideline for Testing of Chemicals 203 and 210.
96-h value of LC_{50}s of adult fish compare to fish embryos were following: acetochlor 0.37 mg/L and 0.61 mg/L; acrylamide 59.0 mg/L and 160.0 mg/L; benzene 560 mg/L and 320 mg/L; colchicine 18.5 mg/L and 39 mg/L; diethylene glycol 45000 mg/L and 30000 mg/L; diethylnitrosamine 560 mg/L and 1200 mg/L; methanol 22300 mg/L and 290 mg/L; Triton X-100 13 mg/L and 17 mg/L.
Results of tested substances were comparable for 7 of 8 chemicals for both life fish stages (adult organisms and embryos in eggs). Our toxicity data on *Danio rerio* eggs showed comparable results and confirmed their alternative use in acute toxicity tests.
This method showed following advantages:
- 100 to 250 fold lower quantity of compounds can be used
- the capacity of the laboratory for the biological testing can increased cca 2000 times
- the method enables to use acute toxicity along with teratogenicity

Keywords: zebrafish, acute toxicity, early life stages, chemicals

Poster

Monitoring cell – cell interaction and the anti-inflammatory activity of glucocorticoids with a new full thickness skin model

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The ability to permit cross-talk between dermal fibroblasts and epidermal keratinocytes is a main advantage of three dimensional full skin models versus monolayer cultures. Both cell species can influence each other, triggered responses correlate closely to *in vivo* monitored responses. For the evaluation of the anti-inflammatory activities of gluco-

corticoids and direct monitoring of pro-inflammatory cytokines we used a new full skin model. Primary fibroblasts and keratinocytes were isolated from foreskin of boys less than four years of age. They were seeded on a collagen matrix (Phenion, Frankfurt, Germany) and cultivated according to common protocol. A stratified epidermis containing stratum basale, -spinosum, -granulosum and -corneum developed on a fibroblast rich dermis. Release of pro-inflammatory cytokines was induced by irradiating the skin models using 50mJ/cm^2 to 300mJ/cm^2 UVB. The release of IL-6 and IL-8 was measured using commercial ELISA (R&D, Wiesbaden, Germany). The IL-6 and IL-8 concentrations in the cell supernatants increased dependent on the used UVB intensity. To monitor which cells are responsible for the release of the pro-inflammatory cytokines, skin model sections were stained with anti-IL-6 and anti-IL-8 (abcam, Cambridge, UK). Both cytokines were predominantly located in the region of vital keratinocytes. Furthermore we could show that Betnesol®, topically applied, generated comparable results to systemic treatment with 20 ((mikro))M Betamethason-17-valerate. In both cases the UVB induced release of IL-6 and IL-8 and their basal levels were suppressed. These results suggest that full skin models offer the opportunity to investigate glucocorticoid mediated anti-inflammatory effects *in vitro* including topical application. Apart from analysing supernatants, they enable IHC studies on tissue that resembles human skin.

Keywords: full thickness skin model, cytokines, UVB