3R = BETTER SCIENCE

20 years jubilee of the SGV and the 3R Research Foundation

Joint Meeting
SGV – 3R Research Foundation - VAWV/IGTP

3 – 4 September 2007
University of Zurich-Irchel,
Institute of Laboratory Animal Science / Institut für Labortierkunde.


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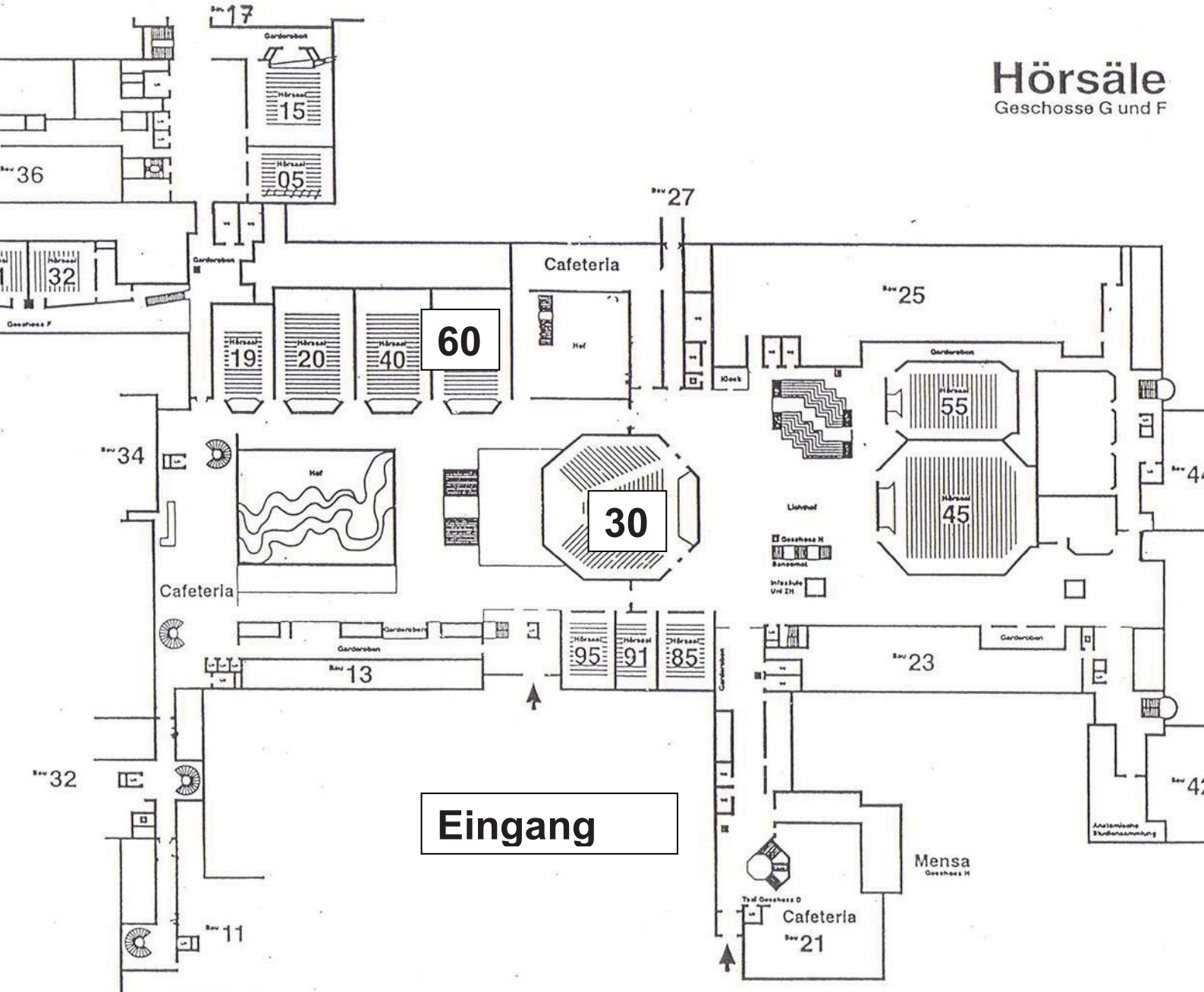
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20th Anniversary: SGV-VAWV-3R Research Foundation

Annual meeting 3 – 4 September 2007 – University of Zurich-Irchel, Institute of Laboratory Animal Science / Institut für Labortierkunde.

Program: 3R = Better Science

	Day 1 ***Humane Endpoint - Stress, Pain Assessment, Scoring***		
8:30 - 9:05	**Registration / Registrierung** **Introduction, Organization / Einführung, Organisierung** (Philippe Bugnon) (Lecture Hall 30)		
9:15 (40 min)	Plenary Lecture A - Plenarvorlesung A SGV-VAWV (Lecture Hall 30) **Humane endpoints as part of the 3Rs** (Coenraad Hendriksen)		
Session 1	**SGV English** (Lecture Hall 60)	**VAWV deutsch** (Hörsaal 30)	
10:00 (30 min)	Lecture 1.1 SGV **Score sheets** (Regula Vogel)	Vortrag 1.1 **CO_2 – Tötung** (Silke Corbach)	(Räume gemäss spez. Plan) Workshop I (auf Deutsch) **Abbruchkriterien**
10:30 (30 min)	Lecture 1.2 SGV **Criteria for HE: cancer research** (Merel Ritskes-Hoitinga)	Vortrag 1.2 **Weitere Tötungsmethoden (exkl. CO_2)** (Ursula Moser)	
11:00	Coffee break – Kaffee Pause		
Session 2			
11:30 (30 min)	Lecture 2.1 SGV **Criteria for HE: infectiology** (Klaus Cussler)	Vortrag 2.1 **"Gentling" bei Ratten** (Pia Seethaler)	Workshop II (Deutsch/Englisch) **Score sheets** (Beat Riederer)
12:00 (30 min)	Lecture 2.2 SGV **Criteria for HE: surgery and transplantation** (Philipp Dutkowski)	Vortrag 2.2 **Handlingstraining bei Primaten** (Michael Keller)	
12:30 (30 min)	Lecture 2.3 SGV **Criteria for HE: brain research** (Lisa Schnell)	Vortrag 2.3 **Vor und Nachteile von Enrichment** (Marika Eschke)	
13:00	Lunch		
Session 3			
14:00 (30 min)	Lecture 3.1 SGV **Endpoint: what takes place after humane endpoints ?** (Huw Golledge)	Vortrag 3.1 **Schmerzerkennung bei Mäusen** (Margareta Arras)	Workshop III (auf Deutsch) **CO_2 Euthanasie** (Silke Corbach)
14:30 (30 min)	Lecture 3.2 SGV **Criteria for HE: genetically modified animals** (Kurt Bürki)	Vortrag 3.2 **Stressreduzierung bei chronischer Belastung** (Klaus Militzer)	
15:00 (30 min)	Lecture 3.3 SGV **Short presentations of posters following by a poster session** (Beat Riederer: Poster Committee)	Vortrag 3.3 **Optimierte Narkosen bei Nagern** (Anja Osterkamp)	
15:30	Coffee break		
Session 4			
16:00 (30 min)	Lecture 4.1 SGV **Criteria for HE: toxicology** (Helmut Schmid)	Vortrag 4.1 **Artgerechte Zucht und Haltung von Marmosets** (Walter Stamm)	Workshop IV (auf Deutsch) **Nützen und Schützen:** Argumentationshilfen (Heinz K. Müller)
16:30 (30 min)	Lecture 4.2 SGV **Criteria for HE: irradiation** (Nicolas Dudoignon)	Vortrag 4.2 **Stressreduzierung bei Kaninchen** (Flora Nicholls)	
17:00 (30 min)	Lecture 4.3 SGV **Discussion about humane endpoints** (all speakers)	Vortrag 4.3 **Weniger Tiere-Verbesserte Zuchtmethoden** (Thomas Rülicke)	
17:30	**SGV Award + Poster Award**; General Assembly	Filmvorführung **"Göttinger Tierpfleger"** (M. Radtke)	
18:15-19:15 20:00	Aperitif, kindly offered by Interpharma a sponsor of the 3R Research Foundation Dinner in Town, Hotel Widder (with registration only); see map in the program booklet - Abendessen im Hotel Widder (nur mit Anmeldung); siehe Plan im Programheft.		

20th Anniversary: SGV-VAWV-3R Research Foundation

Annual meeting 3 – 4 September 2007 – University of Zurich-Irchel, Institute of Laboratory Animal Science / Institut für Labortierkunde.

Program: 3R = Better Science

	Day 2 ***Stiftung Forschung 3R (Lecture Hall 30)*** ***3Rs affect animal welfare, experimental design and results***	
Session 5	**20 years supporting the 3Rs: past and future**	
8:30 (10 min)	Lecture 5.1 **Welcome, 20 years 3R Research Foundation** (Peter Maier and Hugo Wick)	
8:40 (10 min)	Lecture 5.2 **Animal Experimentations, a political issue** (Christine Egerszegi)	
8:50 (30 min)	Lecture 5.3 **NC3Rs – UK initiative to promote good science and animal welfare** (Vicky Robinson)	(Räume gemäss spez. Plan) Workshop I (auf Deutsch) **Abbruchkriterien** (09:00 – 10:00)
9:20 (40 min)	Plenary Lecture B **Implementation of Refinement and Reduction** (Merel Ritskes-Hoitinga)	
10:00	Coffee break	
Session 6	**3Rs benefit animal welfare and affects quality of sciences**	Workshop II (auf Deutsch) **Score sheets** (10:30 – 12:30) (Beat Riederer)
10:30 (30 min)	Lecture 6.1 **Environmental standardization reduces the validity of animal experiments** (Hanno Würbel)	
11:00 (30 min)	Lecture 6.2 **In silico prediction of the toxic potential of drugs and environmental chemicals** (Angelo Vedani)	
Session 7	**Noninvasive methods and benefits for research and animal welfare**	
11:30 (30 min)	Lecture 7.1 **Non-invasive imaging techniques: applications in animal experimentation** (Markus Rudin)	
12:00 (30 min)	Lecture 7.2 **Contribution of MRI to the 3Rs** (Nicolau Beckmann)	
12:30	Lunch	
Session 8	**Organ specific research in vitro**	Workshop III (auf Deutsch) **CO_2 Euthanasie** (13:30 – 14:30) Silke Corbach
13:30 (30 min)	Lecture 8.1 **Investigations on brain infections: Opportunities and limitations of slice cultures** (Stephan Leib)	
14:00 (20 min)	Lecture 8.2 **Designing an accurate three-dimensional blood-brain barrier model** (Lara Ogunshola)	
14:20 (20 min)	Lecture 8.3 **Skin Sensitization: Toxicity testing of cosmetics *in vitro*** (Pierre Aeby)	
14:40	Coffee break	
Session 9	**Replacement, opportunities and limitations**	
15:10 (20 min)	Lecture 9.1 ***In vitro* culture methods for different life cycle stages of the parasite *Neospora caninum*** (Andrew Hemphill)	Workshop IV (auf Deutsch) **Nützen und Schützen:** Argumentationshilfen (15:10 – 16:10) (Heinz K. Müller)
15:30 (20 min)	Lecture 9.2 **Non-mammalian hosts for the study of bacterial infections** (Pierre Cosson)	
15:50 (20 min)	Lecture 9.3 **Quality Control of Production Batches: *In vitro* Testing of Calcitonin Potency and Pyrogens** (Peter Bruegger)	
16:10 (30 min)	Plenary Lecture C **Outlook: 3Rs in safety assessment in pharma industry** (Laura Suter-Dick)	
16:40 (40min)	Plenary Lecture D **Outlook: 3Rs in safety assessment of chemicals in Europe** (Thomas Hartung)	
17:20 (10 min)	**Farewell, Confirmation Cont. Education** (Peter Maier, Philippe Bugnon)	

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SGV = Schweiz. Gesellschaft für Versuchstierkunde (Swiss Laboratory Animal Science Association)
http://www.sgv.org

VAWV = Verein für Aus- und Weiterbildung in der Versuchstierpflege
http://www.vawv.ch

SF3R = Stiftung Forschung 3R (3R Research Foundation)
http://www.forschung3r.ch

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3. September2007: Humane Endpoint - Stress, Pain Assessment, Scoring

Plenary lecture A

Humane endpoints in biomedical research and testing

Coenraad Hendriksen[1, 2] and Iris Boumans[2] , [1)] Netherlands Vaccine Institute (NVI), Bilthoven (NL) & [2)] Netherlands Centre Alternatives to Animal Use (NCA), Utrecht University, Utrecht (NL)
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Adverse effects, resulting in severe pain or distress can accompany animal experiments. If these effects are unexpected, continuation of the study should only be allowed in exceptional cases. Generally, however, effects are inherent to the procedure(s) used and terminating the experiment prematurely would confound with the experimental objectives and results in a waste of animals. Nevertheless, it is equally unacceptable to extend animal distress beyond the point required to meet the scientific objectives. Humane endpoints are limits placed on the amount of pain and distress any laboratory animal will be allowed to experience within the context of the scientific endpoints to be met (Wallace, 2000).
Humane endpoints are particularly relevant for those areas in biomedical research and testing which are characterised by a high percentage of experiments with severe pain and suffering for the animals involved, such as toxicity testing, quality control of vaccines and cancer research. For potency testing of several human bacterial vaccines we have evaluated endpoints that might allow scientific objectives to be met while avoiding significant animal pain and distress. These included the use of clinical signs, pathophysiological parameters such as body weight and body temperature, and also early physiological endpoints as antibody response. This presentation will discuss some of the results.
Most pharmacopoeia's now allow for the use of humane endpoints although their implementation might be frustrated for various reasons. An overview of these obstacles will be presented. One of the major obstacles might be the lack of training of those responsible for the care of the laboratory animals. Particularly observation for clinical signs might be biased by subjectivity and result in increased variability of test results. We recently developed the CD-ROM 'Humane endpoints in laboratory animal experimentation'; an interactive program for educational and training purposes intended for animal welfare officers, researchers, animal technicians and members of animal ethics committees. The CD-ROM contains information on normal behaviour of mouse and rat, on pain and distress, on pathology, on general and specific clinical signs and on humane endpoints. More than one hundred images and video clips are included. The CD-ROM is available free of charge and can be obtained by sending a mail to: i.boumans@vet.uu.nl

Wallace J. (2000) Humane endpoints and cancer research. ILAR Journal, 41, 2: 87-93.

Session 1 SGV

Lecture 1.1

Score Sheets: A Tool of Good Experimental Practice

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The main goal of animal welfare legislation concerning animal experiments in the different countries is the limitation of pain, suffering, injury or fear for the animals involved. Pain and suffering shall only be inflicted on an animal if the purpose of the experiment can be achieved in no other manner. Humane endpoints are essential and must be defined from scientific and welfare points of view before starting a project. Evaluations of the clinical and behavioural status of animals have to be carried out regularly, complying with defined quality standards and record requirements. Furthermore, such records are basic data for the assessment of the degree of severity for statistical and transparency reasons.

Score sheets shall consist of the following elements which need to be discussed and designed by the study director for the species concerned and the particular animal model or method: a) parameters to be observed (with minimal disturbance of the animals, parameters for the evaluation of the general condition need always be selected), b) description of deviations for all parameters, c) frequency of observation (initially, and in case deviations are observed) d) determination of humane endpoints in relation to the scores e) a suitable documentation lay-out for use in the animal facility (date, identification of group of animals, visum).

Score sheets are essential for the following type of procedures: a) in long-term experiments, b) in surgically complicated animal models, c) in experiments causing severe stress, d) for technically complex evaluations, e) in animal models leading to paralysis, severe inflammation etc., f) in new models with minimal knowledge concerning pain and distress.

For an appropriate scoring of animals their normal behaviour and physiological data as well as alterations from the norm have to be well known by the personnel involved. To summarize, score sheets are an important tool, firstly to recognize pain and distress of animals in order to be able to euthanize suffering animals as soon as possible within the experimental context. Secondly, from a scientific point of view score sheets help to obtain clear data. Those are vital for the interpretation of experimental findings for the benefit of scientifically valid results.

Session 1 SGV

Lecture 1.2

HUMANE ENDPOINTS IN CANCER RESEARCH

Merel Ritskes-Hoitinga (1), Bart Savenije (1), Lene Rud (2);
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Humane endpoints (HEP) can be defined as the point at which an animal's pain and/or distress is terminated, minimized or reduced, by taking actions such as euthanizing the animal, terminating a painful procedure, or giving treatment to relieve pain and/or distress (Canadian Council on Animal Care, 1998). Humane endpoints focus on the animal welfare by avoiding unnecessary suffering. At the same time, it is important that studies are carried out until scientifically valid and meaningful results are obtained, in order to avoid that experimental animals have been used unnecessarily. It is the challenge to obtain meaningful scientific data while at the same time safeguarding the animal's welfare.

In experimental cancer research, no specific tumour markers are known to detect liver metastases in humans or animals. General guidelines for HEP in experimental cancer research have been formulated by the United Kingdom Coordinating Committee on Cancer Research (UKCCCR, 1997), and include a loss of body weight of 20% or more, and a tumour burden exceeding 10% of the animal's body weight. At the University of Southern Denmark studies on HEP in rat models on liver metastases of colon cancer have been performed. Cells of the CC531 cell line were injected into the portal vein of syngeneic WAG/Rij rats (250 g of body weight at the start) in two experiments lasting 6 and 9 weeks respectively, and animals were observed closely in order to detect parameters that could be useful as HEP. Laparoscopic examinations at two-week intervals (Kobaek-Larsen et al., 2004) were successfully used in order to be able to follow tumour growth accurately, from day 21 after tumour induction. Individual animals were euthanized when the estimated total tumour size exceeded 3 cm, following the UKCCCR guidelines. Detailed clinical observation and body weight measurements showed no differences between animals with and without liver tumours. Liver enzymes ALP, AST, and ALT in blood were monitored as indicators of liver function.

No indication of pain and/or distress was observed, which may imply that these were not present, and/or out methods were not sensitive enough. In rats carrying liver metastases, mean blood values of AST and ALT showed significant increases, but there was not a clear correlation between individual metastases sizes and these blood values. At this stage they are difficult to define for use as a HEP in future studies. Besides liver enzymes AST and ALT, telemetry (behaviour, blood pressure, heart frequency) and video-recordings of behaviour will be monitored in future studies, in order to evaluate whether pain and/or distress occur. Markers of tumour growth in the blood will also be followed and related to findings from laparoscopic measurements. As yet, imaging techniques are not sufficiently sensitive to detect small liver metastases, but these technologies improve fast and can be expected to be useful to determine HEPs in the future. It is the expectation that a specific combination of critical parameters will form the scientific basis for clearly defined HEPs in this rat model for liver metastases, but further research is needed to elucidate suitable parameters.

Session 1 VAWV/IGTP

Lecture 1.1

Einsatz von CO_2 zur Tötung von Labormäusen

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Immer wieder flammt die Diskussion auf, ob Kohlenstoffdioxid (CO_2) eine geeignete Tötungsmethode für kleine Labornager ist. Gerade Labormäuse werden häufig mit dieser Methode getötet, obwohl bisher kaum wissenschaftliche Untersuchungen zur Tötung dieser Spezies mit CO_2 veröffentlicht wurden.
Die stark reizende Wirkung hoch konzentrierten CO_2s ist bekannt. Dennoch: Das gleiche Gas hat in niedrigen Konzentrationen narkotische Eigenschaften. Aber nicht nur die Konzentration, sondern auch die Art der Applikation hat einen großen Einfluss auf die Wirkung und Wahrnehmung von Kohlenstoffdioxid.
Ein Strömungsmodell, das kann die Vor- und Nachteile der einzelnen Methoden der Zuleitung zeigen, wird vorgestellt. Mit Hilfe dieses Modells konnte ein Deckel entwickelt werden, der eine gute Durchmischung von eingeleitetem Gas und im Käfig befindlicher Luft bewirkt und so eine geringe Anfangskonzentration von CO_2 herstellt. Zusätzlich bietet der Deckel erhebliche Vorteile für das stressfreie Töten von Mäusen.
In einer Studie wurde die Tötung von Labormäusen mit CO_2 hinsichtlich der Tierschutzgerechtigkeit untersucht. Dabei wurden 2 verschiedene Käfigtypen (Makrolon® Typ 2 lang und 3) und verschieden Flussraten berücksichtigt.
Die Studie kommt zu dem Schluss, dass die Tötung mit langsam ansteigenden Kohlenstoffdioxid-Konzentrationen im Gegensatz zur der Tötung mit vorgefüllten Behältern als tierschutzgerecht angesehen werden kann. Denn auf diese Weise kann die narkotische Wirkung des CO_2 in Erscheinung treten. Tiere, die mit der untersuchten Methode getötet wurden, zeigten kein Verhalten, welches auf Schmerzen oder übermäßigen Stress schließen lässt.

Session 1 VAWV/IGTP

Lecture 1.2

Die BVET-„Tötungsrichtlinie“ im Angesicht der Revision der Tierschutzverordnung.

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Aus der laufenden Revision der Tierschutzverordnung ergibt sich ebenfalls Handlungsbedarf für eine Revision der Richtlinien und Informationen zu Tierversuchen und Alternativmethoden, des sogenannten„blauen BVET-Ordners“, der aktuell nur noch online zur Verfügung steht. Die meisten der Richtlinien und Informationen stammen aus der Zeit anfangs der 90er Jahre und benötigen eine Überarbeitung und Anpassung um wieder den neuesten wissenschaftlichen und praktischen Erkenntnissen Rechnung zu tragen.

Eine der am dringlichsten anzupassende Richtlinie ist diejenige über das fachgerechte und tierschutzkonforme Töten von Versuchstieren (BVET-Richtlinie 800.116-3.01). Im Vortrag wird ein Überblick über die bestehende Richtlinie gezeigt. In interaktiver Form soll unter Einbezug des Publikums, welches vorwiegend aus dem Personenkreis der Versuchstierpflege - also Praktiker/innen - besteht, dargestellt werden, in welchen Fragestellungen Änderungen oder Anpassungen nötig und allenfalls zu diskutieren sind.

Session 2 SGV

Lecture 2.1

Criteria for Humane Endpoints: Infectiology

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Animals have been used already for a very long time but it was the development of microbiology in the 19th century which resulted in an enormous increase in the use of laboratory animals. Due to Koch's postulates which stated that the pathogenicity of micro-organisms could be proven by successfully infecting healthy, susceptible animals an intensive search started to find the best suited laboratory animal model for the various infectious diseases.

Today animals are still needed to understand infectious disease processes and to develop effective treatment and prevention strategies. Many regulatory tests for vaccines and anti-infective drugs include challenge procedures in laboratory animals and involve large numbers of animals. Lethality is commonly used as the indicator for the level of protection. For veterinary vaccines it is also necessary to use animals of the target species.

However, animals with induced infections experience significant pain and distress during progression of the disease. Accordingly, it is essential that the discomfort to animals must be limited to what is unavoidable for the conduct of scientifically valuable research.

The presentation will focus on humane endpoint considerations for animal models used in infectiology. Special attention will be given on regulatory tests for vaccine development and quality control. Some examples for the successful use of humane endpoints in laboratory animals and in large animals are highlighted, but also problems to introduce humane endpoints are discussed.

Session 2 SGV

Lecture 2.2

Humane Endpoints: Surgery and Transplantation

Philipp Dutkowski, Department of Visceral and Transplantation Surgery, Swiss HPB Center, University Hospital Zürich
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In the process of developing animal models for diseases, researchers are responsible for the welfare of experimental animals. For the benefit of the animals, they are encouraged to consider the three Rs' as a guideline for their experimental approaches (reduction, refinement, replacement). Careful assessment of the anticipated information obtained in relation to the pain, injury or anxiety of the animals is absolutely mandatory. In this context, three options to meet the 3R criteria in surgical experiments will be discussed:

- Non-invasive clinical screening methods e.g. sophisticated lab analysis or modern imaging techniques provide useful information to follow an experimental course and replace surgical interventions.

- Detailed score sheets with multiple parameters are the basis for maximal refinement in the postoperative course

- an experimental setup with isolated perfused organs reduces significantly the number of animals and experiments. However, the validity of this technique depends highly on the way it is used.

However, in spite of several options for significant improvements, the choice of the animal species is still of decisive importance for later interpretation of the results. Clinical relevance is the final key parameter for surgical research and may only be reached by a model closely adapted to the human situation.
To give an impression on the questions faced in transplantation and surgical research, we will present some experiments to demonstrate where a reduction of animal research is feasible and which questions still rely on animal experiments. Transplantation in particular is a field which has to include many systemic factors, being it the surgical trauma or the graft versus host reaction. The goal of transplantation research is clearly a proof-of-principle in the animal model to demonstrate improvement of surgical/pharmacological strategies, which must undergo rigorous testing in the laboratory prior to transfer to the patient. Transplantation research is attractive to refinement and reduction because each experimental situation requires two animals i.e. a donor and recipient animal.

Session 2 SGV

Lecture 2.3

Humane Endpoints: Brain Research
Where Brain and Heart agonise about a decision

Lisa Schnell
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Our obligation to alleviate the unnecessary pain and distress of experimental animals mandates the exertion of timely euthanasia.
Assessing pain in animals, which is similar to assessing pain in human newborns, relies on the observations of care staff and experimenters. However, one of the notable drawbacks in animals is the interpretation of observations in terms of whether an animal is distressed and in pain.
In the field of brain research, this difficulty is further enhanced by the fact that trauma in the central nervous system (brain and spinal cord) is perceived differently than in the rest of the body. The brain can, as we know, be accessed while a patient is fully awake and some manipulations in the brain do not exert pain at all. Except from occasional headaches, the experimenter has rarely experienced the consequences of brain or spinal cord lesions. An empathetic attitude, which ideally should be displayed, is therefore not easy to acquire.
Equally, for the observers to predict imminent death as an implementation of timely euthanasia is also complex and requires good powers of observation, knowledge of what is normal for that individual animal and strain and species, and good clinical skills. A careful choice of the lesion method is indispensable and can prevent complications like e.g. bladder infections in partially paraplegic rats. These complications, when severe, will lead to an enforcement of euthanasia because of distress of the animal. But even in less severe cases, when euthanasia is not required, scientific outcome may be impaired by the use of therapeutic medication.
We strongly recommend intensive handling and/or training of animals which have to undergo lesions of the central nervous system and the application of the appropriate and least harmful lesion method.

Session 2 VAWV/IGTP

Lecture 2.1

„Gentling“ bei Laborratten: Auswirkungen auf das Verhalten gegenüber dem Menschen

Pia Seethaler, Barbara M. Maurer, Dorothea Döring, Michael H. Erhard
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Einfachste Manipulationen, wie z.B. das Wiegen oder Fixieren, können bei Versuchsratten Furcht auslösen, wenn die Tiere nicht an den Menschen gewöhnt sind. Deshalb ist es zum einen aus Tierschutzgründen, zum anderen im Sinne der Verlässlichkeit von Versuchsergebnissen wichtig, diese Stressreaktionen zu vermindern. In der Dissertation von Barbara M. Maurer wurde untersucht, ob mit Hilfe eines „Gentling“programmes die Furcht von Wistarratten gegenüber dem Menschen reduziert werden kann. Dieses Programm sollte möglichst einfach und praktikabel und damit in Versuchslabors standardmäßig einsetzbar sein. Ziel war es, durch das „Gentling“ (sanftes Streicheln) bei jungen Ratten zu erreichen, dass die Tiere lebenslang gegenüber dem Menschen zutraulicher werden, so dass sie im Umgang mit dem Menschen weniger Furcht zeigen. Um die Auswirkungen des „Gentlings“ zu untersuchen, wurden drei verschiedene „Gentling“verfahren bezüglich ihres Effekts auf das Verhalten gegenüber dem Menschen in komplexen Verhaltenstests überprüft. Das frühe „Gentling“ (4./5. Lebenswoche) wirkte sich auf das Verhalten der Ratten aus und führte zur Furchtminderung gegenüber dem Menschen. Dieser Effekt war beim „intensivierten Gentling“ deutlicher und hielt länger an. Signifikante Ergebnisse bezüglich der „Zahmheit“ der Tiere beim „intensivierten Gentling“ wurden beim Verhaltenstest in der 6. Lebenswoche bis zum Test im Alter von 6 Monaten in jeweils mindestens einer definierten Hauptzielgröße gegenüber der Kontrollgruppe erreicht. Die Untersuchungen zeigen, dass vor allem das „intensivierte Gentling“ nach dem Absetzen einen nachhaltigen positiven Einfluss auf das Furchtverhalten gegenüber dem Menschen hat. Daher kann das „Gentling“ im Laboralltag empfohlen werden, wobei insbesondere die 4. und 5. Lebenswoche genutzt werden sollte.

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Session 2 VAWV/IGTP

Lecture 2.2

Handlingstraining bei Primaten

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Die Belastung der Tiere kann verringert werden, wenn die Haltungsansprüche der Tiere, insbesondere die sozialen Bedürfnisse und der Bedarf an Reizen und Beschäftigung berücksichtigt werden und beim Umgang (Handling) mit den Tieren behutsam vorgegangen wird.
Die Kenntnisse der Bedürfnisse der Primaten bieten das Fundament für eine ethisch vertretbare Versuchsdurchführung.

Es ist sehr wichtig, dass die Tiere durch häufigen und behutsamen Umgang (**Handling**) an den, oder die Tierpfleger gewöhnt werden.
Der Einsatz von Belohnungen kann dabei sehr hilfreich sein.

Durch diese Massnahmen kann der Grad der Beunruhigung und damit die Belastung der Tiere erheblich vermindert werden.

Grundsätzlich sollen Versuchstechniken gewählt werden die das Tier möglichst wenig belasten.
Alles zusammen wirkt sich positiv aus sowohl auf das Tier (geringerer Stress) als auch auf die Versuchsresultate.

Session 2 VAWV/IGTP

Lecture 2.3

Enviromental Enrichment

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Definition von Enviromental Enrichment:
Ist die Verbesserung biologischer Funktionen von restriktiv gehaltenen Tieren, die von Umweltmodifikationen ausgelöst wurden. Eine Verbesserung der biologischen Funktionen beinhaltet z.B. gesteigerte Zuchterfolge oder verbesserte Gesundheit.

Was ist Standardisierung?
Meint im eigentlichen Wortsinn eine Vereinheitlichung von Maßen, Typen, Werkzeugen, Produktions- und Verfahrensweisen durch:

Enviromental Enrichment	Belegungsdichte
Genetik/Zucht	Hygiene
Käfiggröße und –form	Raumklima
Futter / Wasser	Hygiene / Mikrobiologie
Einstreu	Handling

Man erreicht dadurch eine geringe Varianz der Versuchsresultate mit einer möglichst kleinen Tieranzahl.

Wie setzt man Enrichment um?
Es gibt verschiedene Möglichkeiten, den Tieren die Umwelt anzureichern.
Beispiele wären die Förderung der Futtertechnik, in der Raumstruktur oder in der Bereitstellung von Spielzeugarten. Auch die Gemeinschaft von Artgenossen oder die Anwesenheit eines Tierpflegers sind weitere Möglichkeiten. Diese Reiz- und Abwechslungsreiche Umwelt wirkt fördernd auf die neurobiologischen Entwicklungsprozesse und das Verhalten des Tieres.
Da viele Verhaltensmuster der Wildmaus, auch trotz Züchtung zur Labormaus, noch immer erhalten sind, sollte man sich deren Verhalten genauer ansehen. Hieraus können Schlußfolgerungen zur Verschönerung und Anreicherung für den Aufenthalt der Maus im Labor gezogen werden.
Wildmäuse sind Fluchttiere, welche sich in Gegenden aufhalten, die durch Höhlen und Unterschlüpfe strukturiert sind.
Sie leben in Gemeinschaften, zeigen aber ein starkes territoriales Verhalten beider Geschlechter, wobei eine größere Unverträglichkeit bei den Männchen zugrunde liegt bzw. zwischen ihnen besteht.
Die Hauptaktivitätszeiten der Maus liegen in den Dämmerungsphasen.

Das bedeutet für unsere Labormaus:
Man sollte nach dem Absetzen der Mäusejungen stabile Käfiggruppen bilden. Dabei ist zu beachten, wenn es zu starken Rangkämpfen kommt (vorwiegend bei Männchen), diese Mäuse zu trennen, da diese Kämpfe bis zum Tod führen können.

Die Käfige sollten den Normgrößen entsprechen und die Besatzungsdichte sollte nach der Vorschrift der Filasa eingehalten werden.
Der Boden der Käfige sollte mit Einstreu bedeckt sein, da dieser nicht nur die Schadgase wie Ammoniak aufnimmt, sondern die Mäuse es auch zum Graben und Nesterbauen

nehmen. (Nur wenn es das Experiment verlangt, können auch Gitterböden verwendet werden.)
Ein wichtiges Enrichment ist auch das Nistmaterial, wobei Mäuse, welche mit diesem Material aufwachsen, sehr schöne Nester bauen können. Bei Tieren, welche ohne Nistmaterial aufgewachsen sind, ist dieses Verhalten deutlich schlechter ausgeprägt.

Es sollte ein 12 - Stunden – Rhythmus der Hell - und Dunkelzeiten der Tierräume eingehalten werden.

Der Käfig kann mit Röhren, Kisten, PVC, Häuschen usw. strukturiert werden. Dabei sollte beachtet werden, dass dieses Material leicht zu reinigen und desinfizieren ist und beim Zernagen, den Mäusen nicht schadet. Durch einen erhöhten Deckel (Vorwiegend in der Rattenhaltung) können olfaktorische und akustische Abgrenzung zwischen den Käfigen verringert werden.

Die negativen Folgen beim angereicherten Käfigen:
Es kann zu einer erhöhten Territorialität kommen, wodurch starke Bissverletzungen entstehen. Die Hygiene- und Gesundheitskontrolle ist dadurch erschwert. Außerdem kann es hierbei zu einer Erhöhung der Varianz vieler Parameter, z.B. Körper- und Organgewichte kommen.

Die positven Folgen beim angereicherten Käfigen:
Die Gehirnentwicklung der Mäuse mit angereicherten Käfigen ist viel strukturierter als bei Mäusen ohne Enrichment.
Das Wohlbefinden und der Bedarf der Mäuse scheint sich verbessert zu haben.
Bei gesunden Tieren kommt es nach meinem Empfinden weniger zu Stereotypien, was aber wissenschaftlich nicht bewiesen ist.
Reaktionen der Tiere sind stark sex- und stammabhängig!!!

Der Stand der „Enrichment"-Diskussion: *(+ Positiv / - Negativ)*

+	Gitterdeckel	-	geschlossene Deckel
+	Einstreumenge	-	geruchliche Isolation
+	Bodenfläche	-	kleinere Käfige
+	Deckelerhöhung	-	kein Einstreu
+	Nistmaterial- *cave*!	-	Einzelhaltung
+	Gruppenhaltung - *cave*!	-/+	Klettergerüst, „Bauten"
+	„Häuschen" - *cave*!	-/+	Zusatzfutter

Stereotypien:
Sind repetitive, weitgehend invariable Verhaltensmuster ohne offensichtliche Funktion. Es ist ein Indikator einer suboptimalen Haltung.

Sind sie einmal etabliert, sind sie weitgehend unabhängig von Umgebungsreizen und therapeutisch nicht mehr zu beeinflussen. Aktive Tiere neigen eher zu Stereotypien, hingegen passive Tiere eher zu Depressionen. Die Tiere investieren für die Ausführung der Stereotypien erheblich viel Energie und Zeit. Als wesentlicher Faktor für ihre Entstehung wird die Kontrollverlust eines Tieres über seine Umgebung angegeben. Es versucht sich an die Umgebung anzupassen und einen Teil der Kontrolle zurück zugewinnen. Es ist aber nicht hundertprozentig nachgewiesen, dass in einer verarmten Umgebung es zu Stereotypien kommen muss. Denn Mäuse haben ein hohes Maß an routinierten, inflexiblen Bewegungsmustern, deren Abgrenzung zu Stereotypien nicht eindeutig geklärt ist.

Session 3 SGV

Lecture 3.1

Humane Endpoints: what takes place after humane endpoints?

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After a humane endpoint is reached it is our final duty to the animal to give it a good death - the literal meaning of the term euthanasia. As yet however, it is unclear just how humane many ‘euthanasia’ methods actually are.

Carbon dioxide is the most widely used euthanasia agent for laboratory rodents, yet debate continues over whether it may cause pain and/or distress to the animals. Exposure to high concentrations of CO_2 in humans causes both pain and distress so there is good reason to examine whether CO_2 causes similar effects in animals undergoing euthanasia.

Recent experimental work in my own and other laboratories has shed new light on animal welfare aspects of euthanasia with CO_2 by examining its physiological and behavioural effects. During my seminar I will present an update on research examining the humaneness of CO_2 euthanasia. I will examine the various methods by which CO_2 may compromise welfare including pain, behavioural aversion and respiratory effects such as dyspnoea. I will also examine whether different CO_2 application methods (gradually rising CO_2 concentrations versus pre-filled chambers) may avoid some of the potential welfare concerns. My results broadly suggest that whilst pain caused by CO_2 may be avoidable, distress caused by other mechanisms may not.

I will also briefly examine alternative gaseous euthanasia methods including argon and volatile anaesthetics.

Session 3 SGV

Lecture 3.2

Criteria for HE: Genetically Modified Animals

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The majority of transgenic rodent lines do not exhibit signs of impaired well-being. Selected lines however, such as some disease models, develop overt signs of stress and/or pain. For such lines, under the new animal protection act, a permission including a balancing of interests will be required for further breeding of the line. Animals with impaired well-being will have to be restricted to a minimal number needed for experimental purposes. This can usually be achieved by selecting appropriate breeding schemes. For animals developing signs of disease, suffering-reducing measures will have to be used and termination criteria (humane endpoints) will have to be defined.
Given the large number of transgenic lines, the assessment of pain and/or stress in genetically modified rodents will therefore have to be based on relevant, reliable and easy to observe indicators. We have already established and used telemetric and molecular genetic methods to assess pain and distress in the laboratory mouse, but these methods are invasive, technically demanding, expensive and not suitable for routine investigations. We therefore, based on previous work, are evaluating novel screening methods implying a simple and easy to perform observation of the mouse in its home cage. Based on the correlation of pain indicated by telemetric data and behavioural parameters we will establish a score sheet suitable for the assessment of the grade of impairment of the well-being of the animal under observation. The scores will help to determine the humane endpoint for each animal.

Session 3 VAWV/IGTP

Lecture 3.1

Probleme der Schmerzerkennung und -behandlung bei Mäusen

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Folgende Themen werden mit Fokus auf die Labormaus erläutert:

- Schmerzentstehung und –wahrnehmung
- Auswirkungen von (unbehandelten) Schmerzen
- Möglichkeiten und Grenzen der Schmerzerkennung
- Schmerzbehandlung:
    - Häufig eingesetzte Analgetika - Indikationen und Nebenwirkungen
    - Multimodale Schmerztherapie und begleitende Massnahmen zur Schmerzminderung
    - Beispiele verschiedener Behandlungsprotokolle bei chirurgischen Eingriffen

Session 3 VAWV/IGTP

Lecture 3.2

Belastungsreduzierung bei chronischem Stress für Labor- und Versuchstiere

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Stress ist ein Syndrom, das durch heftige Reize, Stressoren, ausgelöst wird und durch eine erhöhte Aktivität des Hormon- und Nervensystems gekennzeichnet ist. Von den Reaktionsstufen im Stress, Alarmreaktion, Anpassung, Erschöpfung, ist vor allem die letzte Form charakteristisch für den chronischen Stress. Zeitlich setzt der chronische Stress nach wenigen Stunden ein, wenn die akuten Stressreaktionen abgeklungen sind, der Stressor aber weiterhin besteht. Chronischer Stress ist durch das Auftreten typischer Organschäden, Thymus- und Hodenatrophie, Herzmuskel- und Nierendegeneration, Vergrößerung der Nebennieren, sowie durch Verhaltensänderungen wie Depression und „erlernte Hilflosigkeit“ gekennzeichnet. Dagegen sind Änderungen bei häufig erfassten physiologischen Merkmalen wie Herzfrequenz, Körpertemperatur, Adrenalin- und Steroidblutspiegel typisch für den akuten Stress. Während also die Vorbereitungsmaßnahmen für die eigentlichen Experimente, - die Haltung in Einzel- oder Fixationskäfigen, Futter- und Wasserentzug, Strafreize beim Training, Injektionen -, für die Tiere einen aktiven Stress bedeuten, führt das Experiment selbst eher zu chronischen Stressbelastungen.
Wesentlichste Entlastungsmaßnahme für Tiere im Experiment ist die perfekte tierärztliche Versorgung einschließlich der konsequenten Schmerztherapie. Der weiteren Entlastung in der chronischen Stresssituation dienen alle Maßnahmen, die dem Tier das Gefühl der Sicherheit und selbständigen Beherrschung von Raum und Zeit geben. Dazu gehört das als „Gentling“ bezeichnete Tolerieren bestimmter Berührungen (z. B. bei Mäusen der Mundregion) und die frühzeitig einsetzende Gewöhnung der Tiere an die Versuchsbedingungen (Anlegen der Verbände und Schutzvorrichtungen, Angewöhnen bestimmter Haltungen, Seitenlage bei Großtieren zur Inspektion). Auch Wahlmöglichkeiten für die Tiere in der Haltung (Enrichment, Temperaturgradienten, „Spiel“materialien) und die Möglichkeit zu Sozialkontakten mit Artgenossen und/oder mit dem pflegenden Menschen können die Belastungen durch chronischen Stress vermindern.

Session 3 VAWV/IGTP

Lecture 3.3

Optimierte Narkosen bei Nagern

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Alle Anästhesieverfahren sollten unter den Gesichtspunkten des Tierschutzes im Sinne der Vermeidung von unnötigen Schmerzen, Leiden und Schäden für das Tier geplant und durchgeführt werden, so sehen es unsere europäischen Richtlinien und Gesetze Sinn gemäss vor. Im deutschen Tierschutzgesetz wird explizit auf die besondere Bedeutung der Verminderung von Schmerzen, Leiden oder Schäden im Tierversuch hingewiesen, Probleme wie Arbeits-, Zeit- und Kostenersparnis dürfen in der Praxis hierfür kein Kriterium sein. So sollten wir zur Anästhesie Methoden und Verfahren wählen, die einen möglichst hohen Grad an Tierschutz und Rücksicht auf die uns anvertrauten Tiere aufweisen. Die stetige Suche nach Optimierung geeigneter Anästhesieverfahren entsprechend dem Stand der medizinischen und veterinärmedizinischen Kenntnisse auf diesem Gebiet muss daher ein zentrales Anliegen aller Beteiligten, die sich im Sinne der „3R“ – reduce (reduzieren), replace (ersetzen), refine (verbessern) – dem Refinement (Verbesserung) verpflichtet fühlen, sein.

Neben der Allgemeinanästhesie gibt es verschiedene Möglichkeiten der Regional- oder Lokalanästhesien, die im veterinärmedizinischen Gebiet neben der mangelnden Kooperation von Tieren auch aus Gründen der Stressreduktion allerdings eher selten praktiziert werden.
Bei Allgemeinanästhesien müssen im jeweiligen Maße die folgenden vier Punkte erfüllt sein:

- Die **Ausschaltung des Bewusstseins (Hypnose)**
- Die zeitige **Analgesie (Schmerzausschaltung)**
- Die **Unterdrückung der Reflexe (Hyporeflexie)**
- Die **Ausschaltung der Muskelspannung (Relaxation)**

Im Vergleich zwischen Inhalations- und Injektionsanästhesie in puncto Sicherheit und Erlernbarkeit steht für uns die Inhalationsanästhesie mit Isofluran an erster Stelle, Nachteil ist der hohe apparative Aufwand.
Die Reduktion von Stress vor der Narkoseeinleitung ist besonders zu beachten. Sie kann durch Verwendung von Sedativa unterstützt werden. Schon simple Maßnahmen während und nach Eingriffen wie z.B. durch mit warmem Wasser gefüllte Gummihandschuhe am Tier oder Lagerung auf Wärmflaschen und andere Wärme-Systeme helfen, die Belastung durch Auskühlung zu reduzieren.
Bei Beachtung dieser Punkte, sowie das Überprüfen und Hinterfragen der eigenen Vorgehensweisen und einem stetigen Streben nach Verbesserung der Techniken, leisten alle Beteiligten einen sinnvollen Beitrag zu den 3 R´s, speziell dem Refinement.

Session 4 SGV

Lecture 4.1

Humane Endpoints in Toxicology

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The role of humane endpoints in toxicological investigations is shortly described and the inherent problem in this area is discussed. In toxicology studies, the adverse effects of chemicals on living organisms are investigated and graduated to allow for a risk assessment. This constitutes an inbuilt dilemma with respect to animal welfare: which degree of adverse effects is acceptable without trespassing against ethical, legal and scientific aspects? The appropriate recognition of pain and distress in laboratory animals is considered to be pivotal to fulfill all demands of the above aspects. A prerequisite for this is a good knowledge of the respective animal species' physiology and normal behavior including the reaction to external stimuli. The practical aspects of assessing the clinical condition of laboratory animals are the cornerstone for decision making whether or not an animal should be humanely killed. There are different approaches to this end, depending on the type of study and the animal species used in the experiment. Measurable (objective) parameters might be used, where appropriate and applicable, as well as physical appearance, clinical and behavioral signs. A useful tool for assessing the health condition is the employment of score sheets which might be general ones or adapted to specific study types. As a general basis the "OECD Guidance Document No. 19 on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals used in Safety Evaluation" (2000) should be taken. Helpful specifications may also be looked up in the animal welfare information papers nos. 1.04 and 1.05 on the pro- and retrospective severity classification of animal experiments issued by the Swiss Federal Veterinary Office.

Session 4 SGV

Lecture 4.2

Criteria for Humane Endpoints in irradiation research

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Human exposure to radiation may induce from slight disturbances to severe injury of multiple organs, potentially life-threatening to the patient. The damage could result from accidental industrial overexposure or medical exposure to radiotherapy. In these cases, one of the most important features is the topography of radiation exposure that directly influences the onset and extent of the clinical symptoms. Thus, the diagnostic, prognostic and treatment may greatly vary from one patient to the other.

In order to provide expertise to medical teams in charge of irradiated patients, our research activities are devoted to the study of radiation-induced damage to the organism. With this perspective in mind, we aimed at setting up experimental models that mimic the diversity of responses of the organism to radiation. For anatomical, physiological or experimental reasons, the studies are performed on several animal species so as to improve our knowledge of the complexity of the phenomenon.

The presentation will focus on the multiplicity of the experimental parameters that modulate the severity of the biological effects and how we are dealing with. Taking into account the complexity and the specificity of the studies, the animal care committee and the scientists are continually putting together their knowledge to improve the welfare of the animals. These efforts help the scientists in their refinement approach when setting up new animal experiments for radiation injury. The main outputs of this tight collaboration were a reference document for the classification of the experiments and the use of score sheets with relevant follow-up criteria and humane endpoints related to our specific research area.

Session 4 VAWV/IGTP

Lecture 4.1

Zentrale Aspekte in der Zucht und Haltung von Marmosets in der Pharmaforschung

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Die Zucht und Haltung von Krallenaffen, zu denen die Marmosets gehören, unterscheidet sich ganz wesentlich von der, anderer Affenarten.
Ein zentraler Punkt in der Zucht ist die Zusammensetzung der Zuchtgruppe, welche aus einem reproduzierenden Elternpaar und deren Jungtieren besteht. Eine Spezialität der Marmosets ist, dass die Aufzucht der Neugeborenen zum grössten Teil durch das Zuchtmännchen und die älteren Geschwister übernommen wird und die Säuglinge nur zur Nahrungsaufnahme der Mutter übergeben werden. Mit dieser Praxis ist gewährleistet, dass das Muttertier, das in der Regel kurz nach der Geburt der Jungen wieder gedeckt wird, ihre Energie nicht für die Aufzucht der Jungen „verschwenden“ muss. Die Anzahl der an der Aufzucht beteiligten Jungtiere sollte 4 Tiere nicht unterschreiten und kann bei genügend grossem Gehege bis 14 Jungtiere umfassen.

Ein weiterer wichtiger Punkt in der Haltung von Marmosets ist eine artgerechte und ausgewogene Fütterung. Das von Futterherstellern angebotene Futter reicht in der Regel nicht aus um die Gesundheit der Tiere längerfristig sicherzustellen darum muss das Futter entsprechend angereichert werden.

Es ist allgemein bekannt, dass Marmosets einen erhöhten Bedarf an Vitamin D3 haben und diesem Umstand ist beim Futterangebot Rechnung zu tragen. Steht den Marmosets Vitamin D3 nicht in ausreichender Menge zur Verfügung, können starke Knochendeformationen bis hin zu Spontanbrüchen die Folge sein.
In der freien Wildbahn sind Baumsäfte ein wesentlicher Bestandteil der Nahrung der Marmosets und diese spielen eine entscheidende Rolle bei der Verdauung der aufgenommenen Nahrung. In der industriellen Haltung können die Baumsäfte durch „Gummi arabicum“ ersetzt werden welches von den Tieren sehr gerne aufgenommen wird.

Session 4 VAWV/IGTP

Lecture 4.2

Stressreduzierung bei Kaninchen

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Kaninchen stammen ursprünglich von den heutigen Wildkaninchen. Das Bedürfnis nach der Lebensweise und dem Sozialverhalten ihrer noch wilden Artgenossen findet man auch bei unseren domestizierten bzw. Laborkaninchen. Soziale Hierarchie, die sich mit Rang- und Territorialkämpfe festlegen lässt, steuert das Aggression bzw. das unterwürfige Verhalten. Die natürlichen Feinde der dämmerungsaktiven Kaninchen (u.a Füchse, Raubvögel) bestimmen das Fluchtverhalten, das sich von meterhohen Sprüngen, Hackenschlagen, in Deckung flüchten bis zum Erstarren ausdrückt. Das Graben von unterirdischen Gängen und Höhlen, die als Schutz vor Feinde und für die Aufzucht der Jungtiere benutzt wird, gehört ebenfalls zur angeborenen Verhaltenspalette.Liebevolle Zuwendung von seitens der Artgenossen in Form von gegenseitigem Lecken, dicht nebeneinander liegen kann innerhalb einer etablierten Gruppe oft beobachtet werden. Das edle Vorhaben für ein möglichst stressfreies Laborkaninchenleben lässt sich leider wegen der experimentalen Bedingungen nicht immer zur vollen Zufriedenheit verwirklichen. (z.B Einzelnhaltung nach bestimmten chirurgischen Eingriffen). Jedoch, etablierte Richtlinien und Empfehlungen über ihre Haltung, d.h. Fläche/Raum pro Tier, Käfigausstattung und -bereicherung, geeignetes Futter liefern bereits einen bedeutenden Beitrag zum Wohl der Kaninchen. Ausserdem kommt der Einsatz des Pflegepersonals, tierärztlicher Diensts und der Forscher, die durch tägliche Verhaltensbeobachtungen, korrektes Handling, geübte Applikationstechniken, sowie durch die Früherkennung von Unbehagen oder Schmerz sofort zu geeigneten Massnahmen greifen sollten. Zur allgemeinen vor allem Stressverminderung gehört eine angepasste Eingewöhnungszeit in der neuen Umgebung. An erster Reihe jedoch, gilt ein frühes Angewöhnen der Tiere an ein liebvolles Handling.
Schliesslich, im Rahmen der Versuchstierhaltung kann eine durchdachte Versuchsplanung im Gespräch mit dem Versuchslaborteams Wertvolles dazu beitragen, den natürlichen und medizinischen Bedürfnisse der einzelnen Kaninchen ohne unnötige Belastung gerecht zu werden.

Session 4 VAWV/IGTP

Lecture 4.3

'Reduction' und 'Refinement' bei der Zucht und Haltung transgener Labortiere

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Im Vergleich zur Arbeit mit klassischen Labortierstämmen, stellt die Zucht und Haltung von transgenen Linien eine Reihe zusätzlicher Anforderungen an die damit betrauten Personen. Diese sollten zum einen über das nötige Wissen bezüglich der Vererbung gentechnisch induzierter Mutationen und der allgemeinen Grundlagen der Labortierzucht verfügen, zum anderen sollten sie dafür qualifiziert sein, spezifische Erfordernisse im Zusammenhang mit der praktischen Zuchtarbeit an transgenen Tiermodellen zu berücksichtigen.

Beim Zuchtziel kann es sich z. B. um den Aufbau neuer transgener Linien, um die Zucht auf Homozygotie für die Mutation, um den Wechsel des genetischen Hintergrundes in Form eines congenen Inzuchtstammes oder um die Zucht von mehrfach transgenen Linien handeln. Die praktische Zuchtarbeit für transgene Linien beinhaltet zusätzlich die Markierung und Genotypisierung der Einzeltiere, das Ansetzen spezieller Kreuzungen, die Beobachtung des Phänotyps hinsichtlich möglicher Belastungen infolge der Mutation und schließlich die detaillierte Dokumentation aller Daten.

Gentechnisch induzierte Mutationen können, ebenso wie spontane Mutationen, neue und z.T. nicht vorhersagbare Phänotypen verursachen. Auch die Gesundheit der Tiere kann beeinträchtig sein, was in manchen Fällen bereits im Zuge der normalen Zucht und Haltung zu einer Belastung der Tiere führt. Aus diesem Grund ist für alle transgenen Tiere eine aussagekräftige Beurteilung des Phänotyps und gegebenenfalls das Ergreifen von Maßnahmen zu einer Entlastung desselben (z.B. durch Anpassung der Haltungsbedingung) notwendig (➔ Refinement). Aber nicht nur die Mutationen selbst, sondern auch Routinearbeiten im Rahmen der Zucht und Haltung transgener Linien, wie die Markierung und die Biopsie zur Genotypisierung, können belastend für die Tiere sein. Hier sind Kenntnisse bzgl. möglichst schonender Methoden gefragt (➔ Refinement).

Die Produktion ungeeigneter Genotypen stellt ein häufig auftretendes Problem bei der Zucht von transgenen Labortieren dar, welches zwangsläufig zur unnötigen Haltung und Tötung von Labortieren führt. Um dies zu vermeiden, ist die Verfolgung einer klaren Zuchtstrategie unerlässlich (➔ Reduction).

Die oben genannten Beispiele machen deutlich, dass die Umsetzung des 3R-Prinzips in Form von ‚Reduction' und ‚Refinement' eine wesentliche Rolle bei der Zucht und Haltung von transgenen Labortieren spielt. Gleichzeitig trägt eine optimale Zuchtarbeit auch hier zu einer Erhöhung der Validität experimenteller Ergebnisse und zu einer größeren Wirtschaftlichkeit bei.

4. September2007

Session 5 3R Research Foundation 20 years supporting the 3Rs

Lecture 5.1

The 3R Research Foundation Switzerland

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The 3R Research Foundation was set up twenty years ago by the Parliamentary Group for Animal Experimentation Questions. The aim of this Group was to bring together the federal authorities, people concerned about animal protection and the pharmaceutical industry to discuss how animal experimentation could be reduced, refined and replaced. The fate of laboratory animals was to be improved without affecting research within the Swiss pharmaceutical industry.

The 3R concept developed by the British scientists Russell and Burch in 1959 was the obvious tool to achieve this aim. Russell and Burch were of the opinion that first-class scientific work inevitably went hand in hand with humane treatment of laboratory animals. Badly planned experiments and incomplete statistics were bound to result in unnecessary suffering on the part of laboratory animals and an unnecessarily high number of animals being sacrificed. The British scientists described their 3R concept – reduction, refinement and replacement of laboratory animals in the life sciences – in *The Principles of Humane Experimental Technique*.

Research into the development of alternative methods is directly connected to the aim of the research. The development of the actual method is rarely the subject of a published article and requires extra backing. For this reason it was essential that the federal authorities and the pharmaceutical industry provide research funds that could be awarded to projects which complied with the 3R principle through the 3R Research Foundation. The Foundation has strict requirements for such projects. They have to meet scientific criteria and be clearly relevant to the 3R principle! Each application is evaluated by a group of experts which includes specialists from the pharmaceutical industry and research as well as people concerned about animal protection. In view of the fact that the Foundation sponsors only true research, the panel of experts cannot guarantee successful results; in true research the result is unknown.

For the past 20 years the collaboration between the pharmaceutical industry and the federal authorities on the funding side, as well as between specialists from the industry, academia and animal protection, has proved its worth and led to results that are relevant to the 3R principle.

Session 5 3R Research Foundation 20 years supporting the 3Rs

Lecture 5.1

The 3R Research Foundation: Achievements and projects

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Over the past 20 years marked changes have been seen not so much in the tasks of the 3R Research Foundation but in how it works, the projects funded and the results obtained. The four main interest groups – academia, industry, animal protection and the authorities – still pursue the common aim of bringing sustainable improvements to animal experimentation through research projects. Updated information about new projects can be found on the Foundation's website at www.forschung3r.ch. Funded projects covers applications in basic life science research, in drug development and safety testing of chemicals and drugs. Successfully completed projects are and will be described in 3R-INFO BULLETINs which are published three times a year and sent out to all those interested. The Foundation's jubilee year was used to design and published a new brochure about the 3R principles for interested lay people. Furthermore in conjunction with ALTEX, a special issue was produced about funded projects in the past and projects in progress. It will increase awareness of the projects within Switzerland and abroad and demonstrates the achievements made in the past regarding the 3Rs. The multi-disciplinary projects will help to inspire young researchers to develop the 3Rs in their particular research area. The Foundation is also part of the European network. In 2002 it was one of the founding members of *ecopa*, an umbrella organisation which brings together national consensus platforms from all over Europe. After 20 years the question arises as to whether the Foundation has helped to ensure that only necessary, well planned and pertinent animal experiments are carried out today? The number of laboratory animals used in Switzerland decreased steadily up until 1998. In the meantime a marked expansion in basic research, in the life sciences, at Swiss universities and in the pharmaceutical industry has been seen. In view of this, the answer must be yes, although there are no statistics on experiments that have *not* been carried out. The increase since 2001 is lower than could be expected from the growth in biomedical research.

Session 5 3R Research Foundation 20 years supporting the 3Rs

Lecture 5.2

Animal Experimentations, a Political Issue

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Frau Nationalrätin Christine Egerszegi-Obrist ist Vizepräsidentin des Stiftungsrates der Stiftung Forschung 3R
Der Stiftungsrat setzt sich aus neun Mitgliedern zusammen, nämlich einer Vertreterin und zwei Vertretern der parlamentarischen Gruppe für Tierversuchsfragen sowie je zwei Vertretern des Tierschutzes, der Interpharma und des Bundesamtes für Veterinärwesen.

Porträt aus der webseite von Ch. Egerszegi

Christine Egerszegi-Obrist wurde am 29. Mai 1948 geboren. Sie besuchte später die neue Kantonsschule Aarau, erwarb das Primarlehrerpatent und studierte danach an den Universitäten Zürich und Lausanne Romanistik. Mit der Gesangsausbildung an der Musikakademie in Zürich ging ein Herzenswunsch von ihr in Erfüllung. Von 1971 bis 1996 unterrichtete sie als Sprachlehrerin an den Bezirksschulen Lenzburg und Mellingen sowie an der Wirtschaftsschule des Kaufmännischen Vereins Baden.

Ihre politische Laufbahn begann 1984 - mit dem Engagement für die FDP Schweiz. Bereits ein Jahr später wurde sie Vizepräsidentin der FDP-Frauen Schweiz. Seit 1991 zählt sie zur Geschäftsleitung der FDP Aargau. Von 1989 bis 1995 war sie Mitglied des Grossen Rates Aargau. Gleichzeitig war sie von 1990 bis 1998 Stadträtin von Mellingen. Ihre Bereiche als Stadträtin: Bildung, Sozialwesen, Gesundheit und öffentlicher Verkehr. 1995 wurde sie in den Nationalrat gewählt. Und im Jahr 2006/07 ist sie als Nationalratspräsidentin die höchste Schweizerin und als Botschafterin im In- und Ausland tätig.

Politische Schwerpunkte: Gesundheit und Soziales

- Seit 1995 arbeitet die Nationalratspräsidentin und Ständeratskandidatin in der Kommission für Soziale Sicherheit und Gesundheit SGK mit und seit 1999 in der Stabilisierungskommission des Nationalrates

- Seit 2001 ist Christine Egerszegi-Obrist Präsidentin der Subkommission BVG - Bundesgesetz über die berufliche Alters-, Hinterlassenen- und Invalidenvorsorge.

Christine Egerszegi-Obrist ist verwitwet, Mutter zweier erwachsener Kinder und wohnt in Mellingen (AG). In ihrer Freizeit geniesst sie vor allem ihre vier Enkel.

Weitere Infos: www.parlament.ch/homepage/pr-praesidenten/pr-nr-egerszegi-christine.htm

Session 5 3R Research Foundation
20 years supporting the 3Rs
Lecture 5.3

NC3Rs – UK initiative to promote good science and animal welfare

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The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) was established in 2004 by the UK government to support the implementation of the 3Rs across the life sciences. Working with stakeholders in academia, industry, Government, regulatory bodies and animal welfare organisations, the NC3Rs has developed a broad programme of activities across all three Rs.

This presentation will cover the NC3Rs strategy, giving examples of current initiatives in academia and industry and will include discussion of some of the challenges of raising the profile and status of the 3Rs.

Session 6 3R Research Foundation
Replacement and Refinement benefits animal welfare and affects quality of sciences

Plenary Lecture B

The 3R Research Centre:
the challenge of the actual implementation of Refinement and Reduction

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Submitting evidence of a search for the 3R alternatives is a mandatory part of the ethical approval process for animal experiments in most EU countries. A recent survey among biomedical scientists at the Radboud University Medical Centre in Nijmegen, The Netherlands, showed that most researchers conduct a nominal search and only use PubMed or MedLine and ISI Web of Science. Generally, few results that are relevant in the field of the 3Rs are found. Indeed, much knowledge of laboratory animal science isn't available from PubMed and one needs to consult specialist databases for searching as well. Additionally, much knowledge is not available online at all, but resides in textbooks and proceedings, and in the heads of people experienced in working with laboratory animals. Most scientists acknowledge the need for a better search for alternatives and would like to get specialist support for this purpose, as the information on the 3Rs is distributed over many different websites and therefore difficult to retrieve. They look to the central animal facility to provide this support. Also the evaluation of the potential implementation needs specialist knowledge and experience.

The 3R Research Centre was founded at the Central Animal Laboratory of the Radboud University Medical Centre to provide such services. This centre is to provide service in searching for alternatives and support in implementation, to all scientists involved in animal experiments. Because Replacement often requires a long-term strategy, the service is mainly focussing on Refinement and Reduction possibilities. This service will take several forms, both physical and virtual. An information specialist is posted at the Central Animal Laboratory for easy access. The information specialist discusses with the scientist the possibilities early in the planning stage of the research. Potential topics to which the 3Rs can be applied are selected. Then keywords from the study and from laboratory animal science are combined and used in literature searches in a selected set of general and specialist databases. Furthermore, a meta-analysis of the literature can be performed. While lists of databases are published on various websites, guidelines for searching, previous search results, lists of experts, books, videos, CD-ROMs, etc., will be made available in detail on an interactive web site. This web site will also provide data on search progress, discussion and training. The 3R-RC will also take part in the FELASA category C course to make future scientists aware of the need for an accurate search for the 3Rs.

Session 6 3R Research Foundation
Replacement and Refinement benefits animal welfare and affects quality of sciences

Lecture 6.1

VirtualToxLab — In silico prediction of the toxic potential of drugs and environmental chemicals: Validation status and Internet access protocol

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We present a receptor-modeling concept based on multidimensional QSAR (mQSAR) developed at our laboratory for the *in silico* prediction of the toxic potential of drugs and environmental chemicals. Presently, the *VirtualToxLab* includes nine so-called virtual test kits for the estrogen (α/β), androgen, thyroid (α/β), glucocorticoid, aryl hydrocarbon, and peroxisome proliferator-activated receptor γ as well as for the enzyme cytochrome P450 3A4. The surrogates have been tested against a total of 798 compounds and are able to predict the binding affinity close to the experimental uncertainty with only six of the 188 test compounds being calculated more than a factor of 10 off the experimental binding affinity and the maximal individual deviation not exceeding a factor of 15. These results suggest that our approach is suited for the in silico identification of adverse effects triggered by drugs and environmental chemicals. We summarize the current validation status of the models and introduce an Internet access portal, immediately available to selected laboratories. Details can be found under http://www.biograf.ch

Session 6 3R Research Foundation
Replacement and Refinement benefits animal welfare and affects quality of sciences

Lecture 6.2

Environmental standardization reduces the validity of animal experiments

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Mice and rats, which together account for over 90% of all experimental animals world wide, are conventionally housed in small cages that lack key features of their natural habitats. Not surprisingly, conventional housing of laboratory rodents was found to induce a range of abnormal behaviours and other signs of poor welfare (Würbel 2001 Trends Neurosci. 24:207-211). Environmental enrichment may prevent abnormal behaviours and improve animal welfare, but concerns have been raised that it might also disrupt standardization, thereby reducing the precision and replicability of animal experiments. In the absence of evidence, we tested these concerns and found that the welfare of laboratory mice can be improved by environmental enrichment without reducing the precision and replicability of the experimental results (Wolfer et al. 2004 Nature 432:821-822). In fact, concerns that environmental enrichment might disrupt standardization came from a flawed concept of standardization. This concept is based on the - true - finding that experimental results may vary depending on environmental conditions and on the – false - belief that envrionmental standardization will ‘spirit away’ such environment-dependent variation. Thus, increasingly rigorous environmental standardzation will indeed render animal experiments increasingly precise, but at the same time increasingly irrelevant. This has been referred to as the ‘standardization fallacy’ (Würbel 2000 Nature Genetics 26:263), which also explains why replicability of experimental results across labs actually decreases with increasing environmental standardization. Based on computer simulations as well as real data, I will show that environmental standardization increases the rate of false positive results (e.g. in drug screening or behavioural phenotyping studies), whereas systematic environmental variation reduces the rate of false positive results and increases external validity, without the need for larger sample sizes. It is time to abandon conventional rodent cages and environmental standardization, and to replace them by enriched cages and systematic environmental variation. This will be to the benefit of both the animals and the research, thereby contributing to Refinement in the best of meanings of the 3R concept.

Session 7 3R Research Foundation
Noninvasive methods and benefit for research and animal welfare

Lecture 7.1

Non-invasive imaging techniques: applications in animal experimentation

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The classical role of imaging, to provide structural and eventually functional information on an intact organism in a non-invasive manner, has been extended in the last decade. Today, various imaging modality allow the study of molecular events such as visualization and quantification of receptor expression, ligand-receptor interaction, the activation of signal transduction pathways, as well as the metabolic, physiological and/or morphological consequences of these molecular events in an intact biological organism. It is obvious that these techniques have become important tools in modern biomedical research, as tools for basic research but also providing relevant readouts (biomarkers) to characterize pathology and therapeutic interventions. Imaging methods are being used both preclinically and clinically, and many of the specific diagnostic readouts currently developed in animal experiments will be translated into the clinics to the benefit of the patient.
Aside from this translational perspective, non-invasive readouts allow for longitudinal study designs. Disease progression can be monitored in individual animals and the efficacy of a therapeutic intervention can be judged by comparison with baseline data recorded in the same animal prior to treatment. This will, in general, enhance data quality, in particular in the cases, in which biological variability is large.
Non-invasive imaging is contributing to two of the 3R's of animal experimentation: multimodality imaging allows a characterization of an individual (human, animal) at various levels, from the molecular target to its structure and function at a macroscopic level, providing information that would be frequently not accessible using conventional invasive techniques. Translatability to humans and from humans should facilitate modeling the clinical situation with animals. Multiplexed information and translatability should lead to refined models. Longitudinal studies in individuals on the other hand will reduce numbers required in a study.

Modern imaging methods provide morphological, physiological, metabolic, cellular and molecular information in a non-invasive manner. This allows the characterization of fundamental biological

Quantitative assessment of the expression and function of gene products in the intact organism with all regulatory processes in place is of tremendous biomedical relevance. The Animal Imaging Center (AIC) of the University/ETH Zürich at Hönggerberg provides a technology platform for structural, functional and molecular imaging. Technologies provided are magnetic resonance imaging (MRI), positron emission tomography (PET) and optical imaging. The instruments are tuned for non-invasive imaging of rodents (rats and mice). A clear research focus of the AIC is the structural and functional characterization of pathologies of the central nervous system as well as studies of neural repair and plasticity (as Center 4 of the NCCR Neural plasticity and Repair).

Session 7 3R Research Foundation
Noninvasive methods and benefit for research and animal welfare

Lecture 7.2

Contributions of MRI to 3R

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The rapid advance of imaging techniques such as magnetic resonance imaging (MRI) has made them indispensable tools in clinical diagnosis. While the drive for method development was clearly given by clinical applications, because of its noninvasive character, MRI has recently spurred great interest in the context of in vivo preclinical biomedical and pharmaceutical research as well. Thus, clinical approaches became available for experimental studies in small animals as well. The major issues that need to be addressed in animal imaging are the demands for high spatial resolution and the associated issues of improved sensitivity. Nevertheless, the technological status of clinical and animal MRI today can be considered equivalent.

MRI may lead to a significant reduction in the number of animals used for experimentation. Depending on the application, a reduction between 80 to 90% is estimated. Since repeated measurements are feasible, each animal can serve as its own control, thereby reducing the variability of the data. Several examples show that MRI readouts are able to detect pathology- and/or treatment-related changes earlier than other standard, often invasive measures, in models of diseases in small rodents. The duration of experiments can therefore be shortened. Thus, from the point-of-view of animal welfare, MRI is able to contribute to reducing the numbers of animals and refining the experiments. Anesthesia is the primary limiting factor of the technique for preclinical use, and this needs to be carefully addressed in each study protocol. However, as gas anesthetics are commonly used, after an examination small rodents recover fast from anesthesia.

Overall, MRI provides a global picture of the pathological status in the animal model. As this imaging technique is largely available in hospitals, there is potential to address translational aspects from the models in small rodents to the human situation. Along with its ability to contribute to 3R, the link between pre-clinical and clinical applications renders MRI very attractive to in vivo biomedical and pharmacological research.

Session 8 3R Research Foundation
Organ specific research in vitro

Lecture 8.1

Investigations on Brain Infections: Opportunities and limitations of slice cultures

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Infectious diseases of the brain are devastating diseases not only with respect to the high mortality rates but also because they cause life-long disabilities in the survivors. For example, bacterial meningitis has a mortality rate of up to 25% and as a consequence of brain damage, neuro-functional disabilities emerge in up to 50% of the survivors. How infection leads to brain injury remains largely unresolved, but converging evidence suggests that the outcome of bacterial meningitis is determined by both, the pathogen and the host's response to the infectious agent. Brain injury caused by bacterial meningitis prominently affects two regions of the brain, namely the cortex and the hippocampus. Brain damage to the hippocampus is documented in approx. 75% of patients dying from the diseases. This form of brain damage is associated with long lasting learning deficits in the survivors.

The multi-factorial pathogenesis of meningitis involving the interplay between the susceptible brain cell types, the bacterial pathogens and the host's inflammatory reaction limits the majority of the current research activities on meningitis to *in vivo* experimental models. An *in vitro* system would facilitate a reduction of animal use by allowing to screen pathogenetic mechanisms for their relevance, and therapeutic approaches for their potential effects, prior to conducting studies *in vivo*. To this end a membrane-based organotypic slice culture system of rat hippocampus was established. The system could be successfully applied for investigations into infectious diseases of the brain including cerebral toxoplasmosis. Co-incubation of organotypic brain slice cultures with live pneumococci did not induce neuronal apoptosis unless cultures were kept in partially nutrient-deprived medium. Thus, pneumococci per se failed to induce significant apoptosis *in vitro*. In the current study we intend to establish a co-culture system of organotypic brain tissue with stem or progenitor cells. This co-culture system will allow us to screen stem cells from different sources for a potential therapeutic application. Furthermore this approach will allow assessing the stage of differentiation that is best suited for integration into the host tissue. This system may lead to a substantial reduction in the number of animals used, as only approaches that prove successful *in the vitro* system would be considered for further evaluation *in vivo*.

Session 8 3R Research Foundation
Organ specific research in vitro

Lecture 8.2

Designing an accurate three-dimensional blood-brain barrier model - fact or fiction?

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Specific cellular interactions are crucial for proper blood brain barrier (BBB) induction and their alteration during cerebral vascular diseases leads to barrier disruption. Despite the use of many different animal models to study diseases characterised by loss of BBB integrity the induction and maintenance of the barrier is still only poorly understood. This is due in part to the complexity of brain structure and to the lack of good *in vitro* models. Most BBB *in vitro* models developed so far are limited by the 2-dimensional nature of the model, use of only 2 of the three cell types that form the barrier as well as the absence of normal cellular interactions. In this study, we have developed a novel *in vitro* three-dimensional BBB model that more accurately reflects the cellular interactions and signaling mechanisms involved in barrier formation. Our model involves suspension of BBB-specific cells in a three-dimensional matrix that supports tube formation and specific cellular interactions. This system will provide crucial information on specific cellular interactions and signals that promote induction of BBB formation during development and can be readily subjected to different insults to understand BBB disruption. We propose that such a model will have wide applications and provide a unique opportunity to reduce the need for difficult invasive animal experiments. I will present and discuss development of this model in detail and also show how we have utilised it to monitor the effect of hypoxic injury on cell fate and crucial signaling mechanisms.

Supported by 3R Research Foundation Switzerland, Project number 93/04 to O.O.

Session 8 3R Research Foundation
Organ specific research in vitro

Lecture 8.3

Skin Sensitization: Toxicity testing of cosmetics in vitro

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Allergic contact dermatitis (ACD) is a delayed-type hypersensitivity reaction induced by small reactive chemicals (haptens). Currently, the sensitizing potential of chemicals is usually identified on the basis of animal studies, such as the murine local lymph node assay (LLNA). Due to increasing public concern and the adoption of the 7th Amendment to the Cosmetics Directive, the development of in vitro models for predicting the sensitizing potential of chemicals is receiving widespread interest.
In vitro sensitization tests need to resume the complex interactions of a chemical with the different compartments of the immune system. In the first phases, the chemical must penetrate the skin and react with endogenous proteins. Haptenated self-proteins are internalized and processed by immature dendritic cells (DC) that become activated.
Procter & Gamble has initiated multiple research projects in order to develop in vitro assays exploiting our current understanding of the molecular and cellular events occurring during the acquisition of skin sensitization. These approaches concentrate on aspects of chemistry/peptide binding/skin metabolism and allergen induced changes in DC measured at genomic and protein level.
Once validated and combined in a test battery, these different in vitro approaches are expected to provide reliable and biologically relevant methods for the detection of contact allergens and will significantly reduce our reliance on animal tests.

Session 9 3R Research Foundation
Without animals?

Lecture 9.1

Development and applications of in vitro culture methods for different life cycle stages of the intracellular apicomplexan parasite Neospora caninum.

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Neospora caninum is an intracellular apicomplexan parasite and represents an important causative agent of abortion in cattle. Two strategies are currently being envisaged to limit neosporosis in cattle: vaccination of newborn calves with (recombinant) immunodominant *N. caninum* antigens and chemotherapeutical treatment. Due to its considerable economic impact, potential prevention and treatment options for neosporosis are being intensely studied. These investigations are somewhat hampered by the fact that *N. caninum* undergoes a complex life cycle comprised of three invasive stages. Sporozoites represent the end-product of a sexual process that takes place within the intestine of dogs. Oocysts containing such sporozoites are shed with the faeces, and they are infective to cattle upon oral ingestion. Tachyzoites are a largely proliferative, and therefore disease-causing stage, which, upon the onset of immunity, differentiates into a cyst-forming and slowly proliferating bradyzoite stage. In an immuno-competent animal, *N. caninum* infection remains unnoticed, and usually tissue cysts containing bradyzoites are formed, which persist within their host for several years without causing any symptoms. However, at the onset of pregnancy, when the immune response is partially impaired, bradyzoites are reactivated and will infect the placental and fetal tissue, and cause abortion, stillbirth, or birth of impaired offspring. In vitro culture of *N. caninum* has been previously established by infecting mammalian cell monolayers with tachyzoites, and up to recently, the definition of antigens used for immunodiagnostic assays and vaccine development, and the identification of novel anti-parasitic drugs, were all based solely on tachyzoite in vitro culture. Nevertheless, the bradyzoite stage, although quiescent and not directly causing disease, is equally important in that it is responsible for horizontal (ingestion of infected meat by carnivores) and vertical transmission (from dam to foetus). Thus a useful interventional strategy must take into consideration both tachyzoites and bradyzoites. While protocols to generate *N. caninum* bradyzoites in mice had been published, these were rather unreliable and consumed large numbers of animals.

In this project, we have elaborated the methodology to generate *N. caninum* tissue cysts in cell culture. The parasites were subjected to physiological stress conditions, including sublethal concentrations of drugs, temperature shifts, pH-alterations, antibodies, cytokines, toxic radicals, and were then visualized the effects on parasite proliferation. Changes in bradyzoite- and tachyzoite-antigen expression were monitored by immunofluorescence, and electron microscopy was used to analyze the ultrastructure of these in vitro generated cysts. We finally established protocols for the generation of *N. caninum* tissue cysts in murine epidermal keratinocytes, Vero cells and fibroblasts.

Several research groups working on *N. caninum* and related parasites have adapted the method, and the possibility to culture *N. caninum* bradyzoites that form tissue cysts has opened avenues for a number of applications. Several bradyzoite-specific *N. caninum* antigens, some of which are currently being evaluated as immunodiagnostic tools, have been identified, and other antigens have undergone primary evaluation as vaccine candidates. We have further exploited both *N. caninum* tachyzoite and bradyzoite culture systems to screen for compounds with pronounced anti-parasitic activities, and identified two drug classes (non-nitro-thiazolides and new-generation pentamidines) as potentially suitable for further development. Bradyzoite cultures were also employed in attempts to establish an in vitro model for visualization of the sexual stages of the life cycle of *N. caninum* in canine intestinal epithelial cell culture, an idea which is currently being further developed. Thus, besides reducing the number of laboratory animals for research purposes, the in vitro method to generate *N. caninum* tissue cysts has considerably enhanced the scientific progress on neosporosis and related diseases.

Session 9 3R Research Foundation
Without animals?

Lecture 9.2

Non-mammalian hosts for the study of bacterial infections

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Pathogenic bacteria can infect patients and are the agent of many infectious diseases, from tuberculosis to diarrhea. Innocuous bacteria, on the contrary, do not cause diseases. The difference between these two types of bacteria, those that can make us sick, and those that can not, is of course essential. Today it is studied very intensely, in the hope of developing much-needed new anti-bacterial drugs.

To study bacterial infectious diseases and to develop new drugs, it is very often essential to test the ability of a bacteria to cause a disease. For this it is necessary to infect a host, typically a mouse, and to allow the disease to progress. These experiments create serious practical and ethical problems.

We are working on developing alternative systems where mice are replaced by non-mammalian hosts. Our results indicate that a significant part of the research on bacterial virulence could be done in such alternative models. This approach reduces the need for animal experiments, and at the same time allows the development of new experimental tools that should facilitate research in this field.

Session 9 3R Research Foundation
Without animals?

Lecture 9.3

Quality Control of Production Batches: In vitro Testing of Calcitonin Potency and Pyrogens

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Since very long times, men is using animals to get some response about specific questions of the daily life. Some questions have religious or mystical, others very practical background.
One says, that Noah was the first man performing a bioassay when he was sending out a pigeon to look out for dry land after the deluge. After the full synthesis of calcitonin in the laboratory in the late 60tys a bioassay on juvenile rats was used to determine and quantify the substance. This method was optimized and about 1980 it was the official method for the qualitity control of production batches before release to the market. The method is cumbersome and demonstrates high standard deviations. Moreover it needs very practiced staff and special equipment. Therefore in Novartis the search for an easy alternative method not using experimental animals started already in 1989. This research was done in collaboration with the endocrinological institute of the university of Heidelberg. A specific tumour cell line carrying receptors for calcitonin is stimulated with the analyte and a reference substance. After a short incubation time, the cells are lysed and the lysate is checked for its cAMP content by ELISA. The measured values are subjected to a ststistical evaluation to calculate the relative potency of the unknown batch. After the validation of the method according to international guidelines the long march to approve the method at the most important health autorities started. It was successful approved by FDA only in 2005.

Another labourous animal experiment for realease testing of pharmaceuticals used for injection is the so called pyrogen test on rabbits. It was discovered in the quality control labs of Novartis that some pyrogenic contamination could not be detected by the pyrogen test while they caused some adverse effects on the patient. After a time of experimentation a method, now called the monocyte activation test was developed and proved reliable in practice for many years on one specific group of compounds. The method has potential to be an in vitro replacement method for the rabbit pyrogen test. For that purpose an European study with different similar methods was performed in the past. But the outcome of the study was not powerfull and it was partially criticized in a peer review in USA. Novartis and WHO developed further the inhouse method and made a lot of good experiences. A general method to be implemented in the European Pharmacopeia was proposed and published. A working party of experts in the field is presently elaborating a text to be finally implemented in the European Pharmacopeia.

Session 9 3R Research Foundation
Without animals?

Plenary Lecture C

3R in safety assessment in pharma industry

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Preclinical safety assessment is of major importance fort he pharmaceutical industry. This applies to industry, patients, and regulatory authorities. To ensure that molecules have the best possible safety profile, animal experiments are necessary and required during the development of new medicines. However, it is in the interest of the pharmaceutical industry and of society to reduce the amount of animal experiments as much as possible. The main reasons for this are two: on the one hand, there are ethical issues linked with performing animal experiments. On the other hand, animal experiments are generally complex, costly, and time-consuming. Therefore, it would be optimal to achieve sound scientific results involving none or at least less animals. Thus, we work at several levels that address this issue and are related to the 3Rs. Several in silico tools that show good prediction for given toxicity endpoints allow us to replace some types of experiments with sophisticated computerized models. Also, for specific endpoints, in vitro assays with cell lines and/or primary cells can be applied to reach a conclusion regarding the toxicity of a given compound. In addition, new technologies are being broadly implemented to improve the sensitivity and predictivity of safety testing. These new technologies include toxicogenomics that uses gene expression to predict and understand toxic events. Thus, gene expression analysis is a refinement of the toxicology endpoints expected to help characterize pharmaceutical compounds earlier and more accurately.

Session 9 3R Research Foundation
Without animals?

Plenary Lecture D

Outlook: 3Rs in safety assessment of chemicals

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ECVAM was created in 1991 further to Directive 86/609/EEC on the protection of animals used for experimental purposes which prescribes to use alternative methods whenever possible. As a service of the EU Joint Research Centre, ECVAM has pioneered the validation process and became a proactive facilitator for effective animal protection, especially in the field of regulatory toxicology. The field of alternatives is currently driven by the expectations from the 7th amendment to the Cosmetics Directive published in 2003, which foresees to phase out animal experiments completely within 10 years, and the legislation for chemicals (REACH) finalised in December 2006. REACH foresees data requirements for more than 30.000 substances produced at levels above 1 ton per year. Alternative methods shall first be considered throughout the testing and be predominantly used for the largest group of chemicals produced between 1-10 tons per year. ECVAM has been charged in 2005 with the coordination of testing strategy development for REACH finalised in May 2007. At this moment about 170 methods are under validation, about 40 at the final stage of ring trials. ECVAM is promoting the concept of an evidence-based toxicology, which aims to quality control toxicological tests in a structured manner. A series of activities including the initiation of and participation in research projects involving 300 partners and more than 80 million € of funding have put the tailored development and validation of alternative methods on a new scale.

Workshop I

Abbruchkriterien

Max Becker
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Interaktive CD Rom der Netherlands Association for Laboratory Animal Sciences (NVP)

Workshop II

What criteria belong on a score sheet.

Beat Riederer, DBCM, University Lausanne
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In the first part of the workshop we will review and discuss different criteria that belong into a score sheet. Some of the criteria will differ between species and depend on specific experimental set-ups and scientific questions. We will also discuss difficulties to determine when to terminate an experiment. It is hoped that participants participate actively in the elaboration of the ideal standard score sheet and report on experienced difficulties in the application of the score system.One has also to ask the question whether the 3Rs are compatible with humane endpoints? Reduce suffering, refine the evaluation criteria and eventually replace a treatment.
In the second part of the workshop, we will perform a pain assessment in the rat. The audience has to define the degree of pain in rats with and without postoperative analgesia, in a first time, based on the impression, and in a second time by scoring specific criteria.

Workshop III

Einsatz von CO_2 zur Tötung von kleinen Labortieren

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In diesen Workshop wird das Strömungsmodell zur Darstellung der Verteilung von CO_2 in Luft näher vorgestellt.
Außerdem werden wir gemeinsam über die in praxi eingesetzten Kohlenstoffdioxid-Tötungsmethoden unterhalten und Erfahrungen austauschen, praxisnahe Verbesserungsmöglichkeiten und Schwierigkeiten diskutieren.
(max. Teilnehmerzahl: 20 Personen)

Workshop IV

Nützen und Schützen: Argumentationshilfen

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Alle lieben Tiere. Tierversuche mag eigentlich niemand. Das führt jene, welche Tierversuche durchführen, für Zucht, Haltung oder Pflege von Versuchstieren verantwortlich sind, oder die in Firmen und Forschungsgruppen arbeiten, welche Forschung an und mit Tieren betreiben, manchmal in schwierige Situationen. Kaum kommt das Thema auf, im Familien- und Freundeskreis, im Verein, der Partei oder anderswo, sind sie in der Defensive, fühlen sich vielleicht gar an den Pranger gestellt.

Tierversuche sind kontrovers und emotional. Keine Frage. Gerade deshalb sollten jene, welche das Gebiet aus eigener Fachkenntnis und Erfahrung kennen, darüber sprechen können.

Im Workshop IV wollen wir uns mit Argumenten Pro und Kontra Tierversuche auseinandersetzen und diese auf dem Hintergrund der eigenen Erfahrungen diskutieren.

Posters

The advantageous use of novel spleen CD8a+ dendritic cell lines - a valuable explorative tool that potentially replaces thousands of mice.

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The study of dendritic cell (DC) biology is an area where the cellular tools currently available are limited and still relies largely in *in vivo*, *in mouse* experimentation. DC being central to the induction of efficient immune responses, there is growing interest in the elaboration of DC-based strategies for the purpose of both cancer and vaccination therapies. However, the fact that DC represent a trace fraction of immune cells *in vivo* and that there are limited *in vitro* systems of study has greatly hampered advances in DC research, while the Replacement, Refinement and Reduction in animal use remain difficult.

The advantageous use of novel, spleen CD8a+ DC lines is hereby reported.

In recent years, Prof. Hans Acha-Orbea's group has developed a transgenic mouse harboring SV40LgT under the CD11c promoter, leading to DC transformation *in vivo*. Consequently, it has been possible to derive several murine DC lines, predominantly from spleen CD8+ DC tumors. Importantly, the cell lines present a phenotype as well as respond to various stimuli in a manner comparable to freshly isolated spleen DC.

The cell lines are readily cultured *in vitro* over months, maintaining all the tested DC functions, and represent a virtually unlimited source of material for the study of DC biology.

A synthetic comparison between the use of DC line versus mouse is presented, including practical and hypothetical aspects. In addition, an example of a research project – currently ongoing in the laboratory – is given, where the cell lines are advantageously used in order to gain knowledge on a particular aspect of DC biology, namely, cell death induced by microbial stimulation.

These novel DC lines allow for extensive experimentation *in vitro*, screening, testing and optimizing, representing an important explorative tool. It is particularly relevant that ten spleens (ten mice) would otherwise be needed in order to obtain only one million CD8+ DC cells. "Ultimately", knowledge gained from DC lines may require confirmation *in mouse in vivo*, but systematic, large-scale animal use can be avoided. The preferential and productive use of DC lines together with the consequent dramatic reduction in mice use illustrates that "ultimately" is an important 3R of "always".

Posters

New decontamination applications with vaporized hydrogen peroxide

N. Khammo & G. McDonnell*

Vaporized hydrogen peroxide (VHP®) has been widely used for the microbial decontamination of enclosed areas and spaces, including isolators, laminar flow cabinets, cleanrooms, biological safety cabinets and general research rooms/facilities. The antimicrobial efficacy of VHP has been demonstrated and widely published against a wide range of pathogens and environmental microorganisms. However, in addition to biological decontamination, many facilities are equally concerned about chemical (including biological macromolecule) decontamination in these environments. These contaminants include biological contaminants, such as proteins and nucleic acids (e.g., DNA in molecular biology laboratories), as well as pure chemical contaminants such as cytotoxic and other drugs. This poster will describe the use of gaseous peroxide for the effective chemical decontamination, including proteins, nucleic acids and cytotoxic drugs. Hydrogen peroxide can be a very effective biological and chemical decontaminant, but will dramatically vary in effectiveness when in a liquid or a gaseous phase. The mode of action of hydrogen peroxide will be also discussed, based on previous and recent results that highlight these differences.

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Posters

Comparison of Unilateral and Bilateral Embryo Transfer in Mice

Claudia Laschalt[1], Thomas Kolbe[1], Martin J. Wolfsegger[3] and **Thomas Rülicke**[1,2]

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The surgical transfer of preimplantative mouse embryos is a crucial technique for the rederivation of strains contaminated with pathogens and for the generation of transgenic mice. Although it is a standard procedure since decades in many laboratories around the world there are no recommendations regarding the most efficient and most refined technique. Embryo transfers are routinely carried out bi- or unilaterally. A bilateral transfer requires extended surgery and anaesthesia and may increase the general burden for the animal. Especially with regard to the three R's of Russel and Burch (1959) the individual specific technique of a laboratory needs to be revised to minimize physical stress for the animals due to the surgical procedure. Therefore, the aim of this study was to compare unilateral vs. bilateral embryo transfer (oviduct transfer) with regard to birth rate when a specific number of embryos is transferred.

The embryo transfer was performed either with a lateral or dorsal skin incision on day 1 of pseudopregnancy (day of vaginal plug) under Xylazin-Ketamin anaesthesia. 10 to 14 embryos (2 days p.c.) were randomly transferred either to the left, to the right or one half each into both oviducts. Overall, 222 embryo transfers were carried out for routine rederivation of different transgenic lines by two persons in two laboratories.

Based on the outcome of our study we achieved the best results (birth rate and developmental rate of transferred embryos) by using the bilateral embryo transfer. However, the unilateral transfer of an appropriate number of embryos into the right uterus horn is nearly equivalent but would reduce the surgical burden for the surrogate mothers.

The unilateral transfer into the left uterus horn is not recommended due to the significant lower birth rate. Concerning the number of transferred embryos (10 to 14) no effect on the developmental rate of transferred embryos in any mode of transmission was recognized.

Posters

The Sudden Noise Test (SNT) as a screening tool for psychogenetic selection in rats: An alternative to shuttle box (SB) testing for active, two-way avoidance

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Introduction and objective. The acquisition of two-way, active SB avoidance behaviour in rodents has been shown to be strongly dependent upon emotional factors, rather than learning capabilities. This is particularly evident when applying the SB test to the psychogenetic selection of Roman high- (RHA) and low-(RLA) avoidance rats. The major reason for the failure to avoid/escape electric shocks in RLA rats is their pronounced freezing (immobility) response in that test (reviewed by Escorihuela et al., Neurosci. Biobehav. Rev. 19, 353-367, 1995; Steimer and Driscoll, Stress 6, 87-100, 2003). Current regulations concerned with animal experimentation make it increasingly difficult to obtain permission for experiments involving electric shock application. The SB equipment is not readily available in most laboratories, and SB testing is also time-consuming, especially when testing and comparing large numbers of potential breeding pairs, as done for psychogenetic selection. The SNT was developed in our laboratory as a possible, more efficient and less stressful alternative to SB testing. The test is based on the observation that RLA rats freeze for a much longer period of time than RHA rats do in reaction to a sudden noise, such as clapping one's hands.

Methods. Rats are introduced to a new cage containing fresh sawdust and left to explore it for 2 min. The standard stimulus is obtained by letting a 230 g piece of iron pipe drop from a height of 40 cm onto a metal base which is in contact with the cage, producing both a loud noise and mechanical vibrations that are transmitted to the floor of the cage. This realistic, potential danger signal induces an immediate startle and freezing response. After the freezing response, which lasts for a few sec for RHAs and up to 2 min for RLAs, the rat resumes exploratory activity. It is left in the cage for another 3 min after the stimulus, and the number of rearings, before vs after freezing, are counted as a measure of exploratory activity. Fecal boli are also counted at the end of testing. Freezing duration can be measured directly with a stopwatch or calculated from the number of video frames (at a rate of 25 frames/sec) showing total immobility. As there are large differences between the rat lines, a time resolution of 1 sec is sufficient to show statistical significance.

Results. Parallel tests conducted on 40 rats (10 males/10 females from each line) have shown a very good correlation between freezing duration in the SNT and performance in the SB, both for active avoidance (Spearman's $r = -0.5358$, $p<0.001$) and for escape failure ($r = 0.5278$, $p<0.001$). Measurements of plasma corticosterone at the end of the test suggest that the SNT is also much less stressful for the rat than the SB test. This simple test is capable of screening 8 to 10 rats/hr (10 x more than SB testing!). The SNT test could probably be automated, although its current version is perfectly functional and efficient.

Conclusions. We suggest the possibility of using the SNT as a suitable alternative to SB testing. This could be applied to psychogenetic selection of the Roman rat lines, in which case both lines (renamed Roman short- and long-freezing: RSF/RLF) would be expected to maintain their traditional behavioral, physiological and neurochemical characteristics, for which they would be regularly controlled. The SNT could also probably be used in some other animal models where SB avoidance is used as a behavioural end-point (e.g. learned helplessness), or for drug tests. However, in this case, proper validation should be performed by direct comparison between the SNT and SB test before further use.